COOPERATIVE PINK HIBISCUS MEALYBUG PROGRAM
ANNUAL REPORT 2001
Update on Current Work in Imperial Valley, California

Contents:

THE PAST TWO YEARS

During the past two years, over 13,000 properties (6,500 in 2000 and 6,700 in 2001) have been surveyed in Imperial Valley, and the results show that the pink hibiscus mealybug (PHM) has not spread beyond the initial infested area of approximately 100 square miles. To date, the mealybug remains exclusively an urban pest, with commercial crops being unaffected. During this time, parasite insectaries have been established and have produced over 490,000 parasites in 2000 and 350,000 in 2001. In 2000, approximately 400,000 parasites were released locally and in 2001 over 200,000 were released. Differences between production and release values, and production

totals between years occur because parasites must be re-cycled back into the colonies to maintain production, and because there was a shift in resources towards field monitoring and evaluation. Monitoring and evaluation activities determined that these recently introduced parasites have resulted in a 90% reduction of the PHM population over this two-year period.

Despite the occasional difficulties in applying materials to some trees, neonicotinoid insecticides show great potential as effective chemical tools for containing and suppressing PHM within its current zone of infestation in the Imperial Valley. Host plant experiments showed that while hibiscus is a preferred host, PHM would also attack a variety of species including grape and lemon.

PROGRAM GOAL AND COOPERATORS:

The goal of this program is to develop and implement a biologically based sustainable approach to suppression and control of populations of pink hibiscus mealybug (PHM) (*Maconellicoccus hirsutus* (Green)) in Imperial County. To accomplish this, a cooperative PHM management team has been formed, comprised of representatives from the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) and Agricultural Research Service (USDA-ARS), University of California (UC), California Department of Food & Agriculture (CDFA), and Imperial County Agricultural Commissioner's Office (ICAC). Member roles and responsibilities are detailed in APPENDIX E. In addition, a close line of communication with neighboring Mexico is facilitated by the attendance of Mexican officials from Program de Sanidad Vegetal (SAGAR) and Instituto Nacional de Investigaciones Forestales Y Agropecuarias (INIFAP) at all workshops and at each quarterly meeting.

The primary topics addressed during the development and implementation of this PHM management program include an extensive and intensive survey to understand the extent of the infestation and the state of its progressive spread, classical biological control (including: local production, release and monitoring of exotic biocontrol agents) to achieve complete control or reduce PHM impact until other management tools become available, and an assessment of new generation and several old generation pesticide products with an emphasis on finding effective products that have reduced negative impact on beneficial spp. (i.e., biological control agents). In addition, studies are ongoing related to host plant preference, suitability etc.

OVERVIEW:

The pink hibiscus mealybug is native within the boundaries of South Asia or Australasia. Its host range is very large, exceeding over 200 plant species, many of which are important in agriculture and as ornamentals. During the early part of the twentieth century, its range extended into Central Asia and Egypt. In Egypt, it was a very significant pest of several common plant species, including cotton. Initially, a predatory beetle species (*Cryptolaemus montrouzieri* Mulsant) was reared and introduced in large numbers. This provided some short-term relief, however, it was not until several parasitoid species were introduced that high levels of control were sustained.

The PHM appeared first in the Western Hemisphere in Hawaii in 1984, followed by the Caribbean islands of Grenada and Carriacou in 1994. Its range soon included over 25 Caribbean Islands (APPENDIX A). Subsequent to the Caribbean invasion, biological control efforts were implemented in the Caribbean by USDA-APHIS in cooperation with each island country and CABI (Center for Agric. & Bioscience International). USDA-APHIS has referred to their early and continued program involvement as the "Off Shore Project" representing a proactive approach to impending pest invasions into North America. In 1999, the PHM had spread to Imperial County, California, USA and the Central American country of Belize. In 2000, it was present in the Bahamas and northern, South America (i.e. Venezuela/Guyana region).

The PHM was first detected in Imperial Valley, CA during August 1999, following the submission of a mealybug specimen to the Imperial County Entomologist by a homeowner. Population densities of PHM on mulberry, silk oak, hibiscus and natal plum were determined to be high in several communities in southern Imperial Valley . In speaking with several homeowners in Calexico about their infested mulberry trees, they claimed to have experienced the same mealybug problem as early as the summer of 1997. In response to the Imperial Valley infestation, two parasitoid species, *Anagyrus kamali* Moursi and *Gyranusoidea indica* Shafee, Alam & Agarwal, were released at ten infested sites in the fall of 1999. At that time, approximately 3,000 parasitoids of each species from USDA insectaries were released. These initial releases were followed by several additional shipments of parasitoids, used for release and the setup of a PHM parasitoid rearing facility in Imperial Valley, under the direction of the California Department of Food and Agriculture in cooperation with USDA and the Imperial County Agricultural Commissioner's Office. The insectary began producing and releasing large numbers of parasitoids by late June of 2000.

Historically, insecticides and oils have been found to be relatively ineffective in controlling this pest and several other mealybug species as well. In part, this is due to the profuse layer of protective wax that is produced by mealybugs. New generation, systemic products have shown greater promise in controlling mealybugs, and are likely to be most useful in eliminating isolated infestations. Currently, classical biological control offers the greatest opportunity to control this pest as it becomes established in new regions. The biological control program initiated by USDA-APHIS and cooperators has demonstrated a high level of effectiveness throughout the Caribbean region. The potential for success by the available parasitoid species within the arid region of the Colorado Desert was of concern. Since 1999, *A. kamali* has performed very well, with levels of parasitism commonly exceeding 50% and PHM densities are a small fraction of those in 1999.

Pink Hibiscus Mealybug Population Status in Imperial Valley

Intensive Population Survey of the Pink Hibiscus Mealybug in the Imperial Valley

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A survey was developed to determine the distribution of the PHM within Imperial Valley communities and agricultural fields. The plan called for a comprehensive survey of infested and non-infested communities and rural areas from April to October during the years of 2000 and 2001. Last year's report presented a complete summary of results for 2000. In this report, the 2000 survey is briefly reviewed, followed by a full report of 2001 survey results.

Survey Summary 2000

Description:

The 2000 survey was conducted by six personnel. The overall survey consisted of four parts. CDFA's trapping system for mapping the Imperial Valley was used. The system is a regular grid of one-mile square units. The description of each survey component is illustrated in Appendix B1.

1. *Leading Edge:* Based on a broad survey conducted during the fall of 1999, a line was drawn to encompass areas where infestations were present. Following the spring of 2000, this line was moved from just east of Calexico and El Centro, because small populations were found in the town of Holtville and on the Imperial Valley College campus. No change in the leading edge has occurred since. The leading edge survey included all square mile quadrats along the line defining the leading edge. They were surveyed in the spring and again in early fall.

2. *City Survey:* All cities within the leading edge were intensively surveyed in the spring/early summer and again in late summer/fall. The goal was to inspect vegetation at residential and commercial sites in approximately 25% of each community. In addition, communities outside of the leading edge (i.e., Brawley, Westmorland, Calipatria and Niland) were surveyed during mid to late summer, when new infestations were most likely to be detected.

3. *Transect Survey:* Quadrats (one sq. mi. each) within transects radiating out from the leading edge (up to 11 miles) were surveyed from spring to fall.

4. *Delimitation Survey:* Within the leading edge, nearly all fields in production and rural home yards were inspected from May to August.

Results:

The extent and intensity of the 2000 survey is illustrated by the number of one square mile quadrats highlighted in Appendix B1 and map of survey points (Appendix B2). The survey demonstrated that the pest population was confined to Calexico, Heber, El Centro, Imperial, Holtville, Seeley, the Imperial Valley College campus and the Naval Air Base north of Seeley. No areas north of the town of Imperial were found to be infested, nor were any field crops infested. The PHM was distributed throughout Calexico. It was found throughout the small community of Heber and concentrated in a several block area of Seeley. The PHM population in El Centro was mainly concentrated in the southwest part of the city and the population in Imperial was very sparsely distributed.

2001 Survey

The 2001 survey was conducted by four personnel and consisted of three parts. CDFA's grid system of mapping the Imperial Valley in one square mile quadrats was used (Appendix B3). The overall survey was intended to determine, 1) the status of PHM in commercial crops (none were found in 2000), 2) changes in PHM distribution, and 3) changes in infestation levels between years.

Description:

1. *Transect Survey:* This survey was primarily intended to retest the hypothesis that PHM was strictly a yard landscape plant pest in Imperial Valley, and not found on commercial crops. As illustrated in Appendix B3, transects extended from the border of Mexico to approximately 24 mi. north, extending far north of areas known to be infested in 2000. Transects were surveyed from spring to fall. Two edges of 10 fields were carefully inspected per one square mile quadrat.

2. *City Survey:* All cities within the leading edge were surveyed one time. Calexico was surveyed intensively during June in order to compare infestation levels between years (2000 vs. 2001). That is, to compare the percent infested properties in Calexico between the two years. During the later half of summer, communities outside of the leading edge [including: Brawley, Westmorland, Calipatria and Niland] were surveyed as well. The timing of this 2001 survey maximized the opportunity to detect infestations in these communities where no infestations were detected in 2000.

3. *Urban/Agriculture Interface Survey:* The one square mile quadrats surrounding each community were surveyed during the summer. This survey maximized the chance of detecting agricultural field infestations, because these fields were adjacent to urban centers, where the majority of the PHM population exists.

It is noted that rural yards in the delimitation area (i.e., within the leading edge) were not intensively surveyed as they were in 2000. A significant addition to the survey in 2001, included the survey of the Bard/Winterhaven area east of Imperial Valley, near the Colorado River (see Appendix B4). Yards in and near these small communities were inspected for infestations.

Results:

Transect Survey: Over 2,200 sites in commercial fields and over 550 locations representing properties outside of primary urban centers (including: Imperial Valley College campus, Barbara Worth Country Club and a limited number of rural yards) were inspected during the transect survey (Appendix B4). No mealybugs were found in commercial crops.

City Survey: In Calexico, the PHM was detected at 10.2% of the properties inspected with host plants (Appendix B5). This compares to a detection level of 38% during the same mid-late June period in 2000. In El Centro, the detection level was 8.7% in 2001 compared to 15% during the same late August – September period in 2000 (Appendix B6). The ranking of host plants remained relatively constant from 2000 to 2001, however there were several notable changes in infestation levels (Table 1.). The infestation level in silk oak dropped from 44% to 4% . Furthermore, of those host plant species whose infestation frequency was low in 2000, infestations were undetectable or nearly undetectable in 2001. Conclusions should be guarded for those plant species in table 1 that are uncommon, and therefore represented by a small sample size.

Table 1. Apparent plant preferences in the extensively infested town of Calexico, CA.

PLANT	# CHECKED[a]		# INFESTED		% INFESTATION	
	2000	2001	2000	2001	2000	2001
Carob Tree	48	35	26	17	54	48.6
Mulberry	662	408	333	142	50	34.8
Silk Oak	80	65	35	3	44	4.6
Orchid Tree	65	35	26	17	40	48.6
Hibiscus	121	61	41	16	34	26.2
Loquat	9	0	2	0	22.2	0
Grape	37	11	6	0	16	0
Cape Honeysuckle	69	34	11	0	15.9	0
Natal plum	281	230	36	0	12.8	0
Fig	78	36	7	0	8.9	0
Rubber tree	160	96	13	0	8.1	0
Citrus	285	187	10	3	3.5	1.6
Indian laurel	13	445	1	0	7.7	0
Mock orange	18	0	1	0	5.5	0
Pomegranate	49	3	2	0	4.1	0
Roses	193	334	4	0	2.1	0

[a]number of surveyed home sites with that plant species

An additional sampling study of PHM infestations was conducted to characterize population status across years. Twenty-five total sample sites were identified in Calexico, Heber, El Centro and Seeley (12, 4, 5 and 4 respectively). All hosts were mulberry or carob trees. The same 25 trees were sampled in August of 2000 and 2001. Ten terminals were randomly selected from each tree and were scored from 0 to 4 based on the following PHM infestation criteria. The evaluation was conducted by the same individual both years.

0= not present
1= no living stages, only remains from previous activity
2= life stages found with careful examination of terminals
3= life stages visually apparent without handling terminals
4= heavy infestations

The primary emphasis of this study was to sample spatially separate areas, therefore all 4 towns were used in the study. The average ranking in August of 2000 was 1.48 compared to 0.58 in August of 2001. These values were significantly different (Table 2.)

Table 2. Analysis of variance results from year to year infestation rankings of 25 sites in four towns in Imperial Valley.

Source	DF	Type III SS	F	PR>F
Tree	24	351.5720	18.79	0.0001
Year	1	101.2500	129.85	0.0001

Results from this study were highly significant, and strongly supportive of the survey results, showing that populations in the infested cities were considerably lower in 2001.

Urban/Agriculture Interface Survey: The agricultural fields on the perimeter of each community were found to be free of the PHM. Many of the fields were in close proximity to infestations in residential areas, yet no commercial crops were found infested.

Summary of 2001 survey:

The PHM infestation boundary in Imperial Valley has remained static in 2001. Boundaries of the infestation are essentially the same between years. The population of PHM within the two largest, infested cities (Calexico and El Centro) has declined. Populations of PHM in the community of Seeley and the Naval Air Base appeared somewhat more active than elsewhere in the region during 2001 and should be looked at carefully in 2002. Thus far, the PHM remains exclusively an urban pest. Commercial crops have been free of PHM.

Several points regarding the general survey of all towns should be noted.

1. Seeley represents the one location where a modest increase in percent infestation occurred. The increase of 3% is not extensive, but this needs to be surveyed in 2002.
2. The Naval Base still has a troublesome infestation. Although there was a slight decrease in 2001, it needs to be inspected in 2002. Part of the problem in this area was due to the

abatement of mosquitoes on the base. The insecticide Malathion is sprayed on a weekly basis during much of the year. The facilities control unit has been consulted. They will attempt to eliminate much of this spraying in 2002.

3. The town of Imperial represents the northern-most boundary of the mealybug infestation. The two sites found to contain PHM were chemically treated with the intention of eliminating them.
4. Holtville was surveyed extensively in the spring and in late summer of 2001. The infestation was present in the community park in the center of town. The two carob trees will be chemically treated in May or June of 2002.

Acknowledgements.

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Biological Control Project in California
Section I:
Status of Pink Hibiscus Mealybug Biological Control In Imperial Valley (Jan. to Dec. 2001)

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Parasitoids were released soon after the detection of the pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green) in Imperial Valley in August of 1999. Beginning in late September, 6,200 parasitoids (obtained from USDA-APHIS) were released at ten locations during the fall of 1999 to initiate a biological control effort against this pest whose population was in a state of explosive increase. This was followed by plans to rear and release parasitoids locally. Starting in early summer of 2000, local production of parasitoids resulted in the release of 399,000 *Anagyrus kamali* Moursi and *Gyranusoidea indica* Shafee, Alam & Agarwal [family: Encyrtidae] by December of 2000. Releases were made in over 400 residential sites covering all areas in Imperial Valley affected by the pink hibiscus mealybug (PHM). During 2001, these biological control agents were again produced at the El Centro insectary in Imperial Valley, CA. Many of the parasitoids produced were delivered to Mexican cooperators for release in Mexicali Valley, which borders Imperial Valley (Table 1). For 2001, additional biological control activities included a more intensive effort of: 1) monitoring PHM densities and parasitism, 2) monitoring hyperparasitism of the newly introduced parasitoids (by resident hyperparasitoid species), and 3) monitoring non-target effects; examining if any resident/native mealybug species are parasitized by the two newly released parasitoids.

SECTION I a: Rearing and Release

In 2001, over 78,000 *Anagyrus kamali* and 126,000 *Gyranusoidea indica* were produced and released (Tables 1& 2). Mass production was again made possible by the use of Japanese pumpkins (*Cucurbita moschata*, cv. Chirimen) to rear the PHM as host material for the parasitoid species. These pumpkins were grown during the spring and early summer (early February – mid-June) at the Imperial Valley Research Center and at the CDFA, North B St. site in Sacramento from spring through late fall (late April – mid-Nov.). In addition, small numbers of pumpkins were grown in

greenhouse facilities during the winter and spring in Imperial Valley. The low level production of greenhouse pumpkins along with sprouted potatoes, provided the means to keep the cultures in a maintenance mode from February through late April. As described in last year's report, cultures of the PHM and parasitoids were again maintained in facilities consisting of two, 55' trailers modified with double entrances and cabinets to prevent culture contamination.

The *A. kamali* population released from 1999 through 2001 represented a mixed culture originating from China and Hawaii. As of December 2001, an insectary culture consisting of a new population of *A. kamali* from Egypt was initiated based on material received from USDA-APHIS in Mission, TX. This population was obtained (D. Gonzalez, U.C. Riverside) from a climate very similar to that found in Imperial Valley. Therefore, because of its close climatic match, it may provide even better control of the PHM than that obtained by the previously released population. In addition, following satisfactory results from host range studies currently underway at USDA-ARS facilities in Newark Delaware, it is likely that *Allotropa* nr. *mecrida* (family: Platygasteridae) will become available for rearing and release by mid-summer of 2002. This parasitoid was also collected in Egypt.

TABLE 1. **El Centro Insectary Production Of Pink Hibiscus Mealybug Parasitoids in 2001**.

Month	*Anagyrus kamali*	*Gyranusoidea indica*	Total Production
Jan	**15,500**	**14,700**	**30,200**
Feb	**17,900**	**25,200**	**43,100**
Mar	**17,050**	**39,500**	**56,550**
Apr	**9,900**	**15,100**	**25,000**
May	**235**	**1,450**	**1,685**
June	**3,200**	**6,952**	**10,152**
July	**13,500**	**22,450**	**35,950**
Aug	**10,000**	**16,050**	**26,050**
Sept	**14,400**	**13,575**	**27,975**
Oct	**24,050**	**30,350**	**54,400**
Nov	**15,000**	**19,950**	**34,950**
Dec	**1,800**	**4,250**	**6,050**
Total	**142,535**	**209,527**	**352,062**

TABLE 2. El Centro Pink Hibiscus Mealybug Insectary Delivery Records for 2001

DATE	SENT TO	*Anagyrus kamali*	*Gyranusoidea indica*	TOTAL
Jan 01	**Imperial Valley**	4500	1500	6000
Feb 01	**Imperial Valley**	7000	9200	16200
6 Feb 01	**St. Martin, French West Indies**	5000	3000	8000
16 Feb 01	**St. Martin, French West Indies**	800	6000	6800
27 Feb 01	**St. Barthelemy, French West Indies**	1800	1000	2800
Mar 01	**Imperial Valley**	8000	27500	35,500
Apr 01	**Imperial Valley**	2600	1600	4200
9 April 01	**Bahamas**	3000	3000	6000
19 April 01	**Mexicali**	0	2100	2100
23 April 01	**Mexicali**	2000	2800	4800
3 July 01	**Mexicali**	400	7750	8150
12 July 01	**Mexicali**	1550	6000	7550
1 Aug 01	**Mexicali**	8050	9600	17650
8 Aug 01	**Mexicali**	1700	5100	6800
15 Aug 01	**Mexicali**	1300	3600	4900
24 Aug 01	**Mexicali**	1500	3600	5100
30 Aug 01	**Mexicali**	1450	1600	3050
6 Sept 01	**Mexicali**	2600	1600	4200
13 Sept 01	**Mexicali**	2050	2000	4050
20 Sept 01	**Mexicali**	2350	2750	5100
27 Sept 01	**Mexicali**	2650	3025	5675
3 Oct 01	**Mexicali**	3900	8050	11950
11 Oct 01	**Mexicali**	5000	5500	10500
17 Oct 01	**Mexicali**	4000	5000	9000
25 Oct 01	**Mexicali**	4900	3500	8400
TOTAL		**78,100**	**126,375**	**204,475**

SECTION I b: Field Monitoring Results and Discussion:

Pink hibiscus mealybug densities and parasitism in mulberry trees at three residential locations have been monitored since 1999. Three more sites with mulberry trees were added to the list of monitored sites in January of 2000, along with three carob tree (non-deciduous) sites beginning in June of 2000. Samples consisted of the terminal and adjacent 5 full leaves on 8 branches per tree; counting all egg masses, second and third instars, and adult males and females. Four beat cloth samples were taken at each site to assess predator (especially the mealybug destroyer, *Cryptolaemus montrouzieri*) activity.

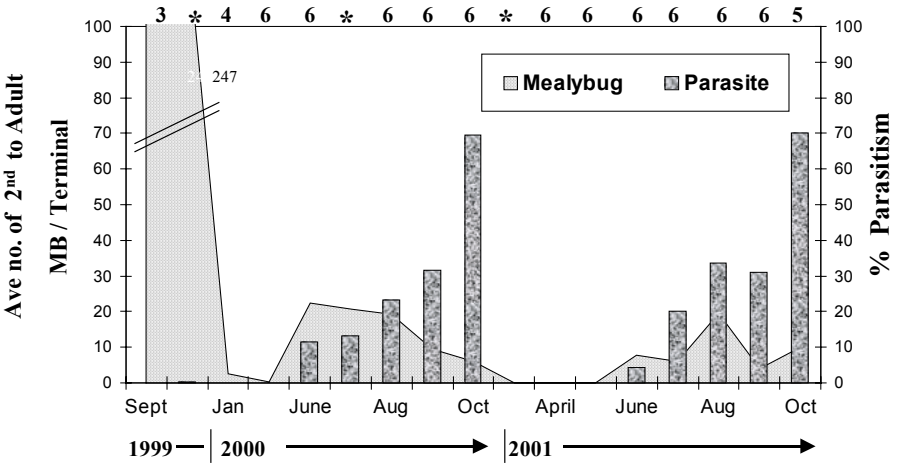
In Imperial Valley, population densities of PHM begin to increase in June, from nearly undetectable levels (Fig. 1). Numbers were somewhat lower overall in 2001 than in 2000. However, what has been most noticeable is that numbers have been dramatically lower for two consecutive years compared to those in the fall of 1999, when mean numbers per terminal were 247 second instar to adult mealybugs per sample. Percent parasitism increased during both years, consistent with increasing summer population densities of PHM, demonstrating a strong density dependent response (Fig.1). Parasitism was difficult to assess prior to June in 2001, because PHM densities were very low. Percent parasitism was variable among sites, however, parasitism levels were similar on mulberry and carob trees; two PHM host plants frequently attacked. (Table 3). The very high levels of parasitism in the October samples may be exaggerated, because they coincide with fall conditions that cause a slowing of PHM reproduction and development, and the characteristic movement of PHM from branch terminals to the bark of large branches and trunks of deciduous trees. Although little is known about how these events influence mealybug and parasitoid activity, they could cause an "accumulation" of parasitized individuals by reducing the mobility of parasitized mealybugs. Percent parasitism is based on mealybugs collected as third instars to adults, prior to becoming mummies. Nearly all parasitism (>95%) was caused by *Anagyrus kamali*. However, elevated levels of *Gyranusoidea indica* have been recorded in the late fall (Nov.- Dec.) of each year, based on corrugated cardboard band sampling of PHM on the bark of large tree limbs. A comprehensive set of figures pertaining to the population monitoring of the PHM and parasitism is presented in Appendix C, illustrating detailed population patterns by sample site.

Hyperparasitism of principally *Anagyrus kamali* by *Marietta* sp. reached a level of 34% by October of 2001. This result was nearly identical to that found in October of 2000 (est. 38%). Hyperparasitism was calculated as the number of hyperparasitoids emerging from a sample divided by the number of hyperparasitoids plus the number of primary parasitoids that emerged. These levels may be biased in an upward direction because nothing emerged from many of the *A. kamali* mummies in many of the samples collected in October. It is unknown whether these parasitoid species (primary and secondary parasitoids) exhibit some degree of winter diapause.

Very few potential predators of the PHM were collected in beat-sheet samples. No *Cryptolaemus montrouzieri* lady beetles were collected.

Fig. 1. Pink Hibiscus Mealybug on Branch terminals of Mulberry Trees

Imperial Valley, California



* PHM not sampled,

Note: # = Sample Size (i.e., no. of sites)

TABLE 3. Percent Parasitism of Pink Hibiscus Mealybug At Ten Release Sites In Imperial Valley.

Site No. & Host Plant	April 2001	May 2001	June 2001	July 2001	Aug. 2001	Sept. 2001	Oct. 2001
4 Mulberry			-	**20** (70)	**75** (4)	**33** (6)	**58** (36)
6 Mulberry			**0** (12)	-	**61** (80)	**0** (14)	
9 Mulberry			-	**7** (100)	**12** (8)	**42** (62)	**41** (29)
9 Hibiscus	**0** (7)	**33** (3)	**25** (4)	**7** (14)	**33** (60)	**52** (124)	-
10 Mulberry			-	**0** (4)	**12** (34)	**28** (36)	**100** (3)
11 Mulberry			**10** (19)	**45** (100)	**60** (73)	**64** (51)	**86** (29)
12 Mulberry			**4** (69)	**6** (50)	**15** (13)	**17** (18)	**65** (20)
19 Carob			**48** (100)	**64** (72)	**26** (3)	**80** (5)	-
20 Carob			-	**0** (40)	**89** (3)	**40** (5)	**66** (6)
21 Carob			**8** (36)	**49** (100)	-	-	-
Mean			**15.8%**	**22.0%**	**42.5%**	**39.5%**	**69%**

*ants were very abundant

** PHM at very low density, few or none collected

SUMMARY

The PHM population densities have stayed low for two consecutive years. Parasitism by *A. kamali* increased greatly as PHM density increased in mid-summer of each year. Parasitism levels commonly exceed 50% and are as high as 90%. Parasitism of resident and native mealybug species by *A. kamali* has not been detected. Peaking at levels around 35%, parasitism of *A. kamali* by resident hyperparasitoids is significant, however, it probably has only a moderate level of impact on *A. kamali*. Populations of PHM at a few sites continue to be noticeably higher than at the majority of locations. Observations have suggested that ants tending PHM are closely link to population buildup.

Acknowledgments:

A special thanks is given to a number of Scientific Aids and others including: Karina Carrera for her diligent release of parasitoids and careful monitoring; James Brian Morse and Josue Vallejo for the initial setup of the insectary and first year production; Jose Zuniga and Jose Aguilar for second year rearing; and Jim Brown (CDFA, SR. Agric. Biotech) and Jose Encalada-Fleytes for their very successful production of Japanese pumpkins in Sacramento and Brawley, for which the rearing of PHM and parasitoids was essentially dependent.

Biological Control Project in California

Section II: Sleeve Cage Evaluation of Parasitoid and PHM Activity in the Imperial Valley CA

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The objective was to identify the active period of PHM and its natural enemies under local conditions found in the Imperial Valley and to assess the natural enemies' (*Anagyrus kamali*, *Gyranusoidea indica*) potential for establishment and control of PHM.

Methods:

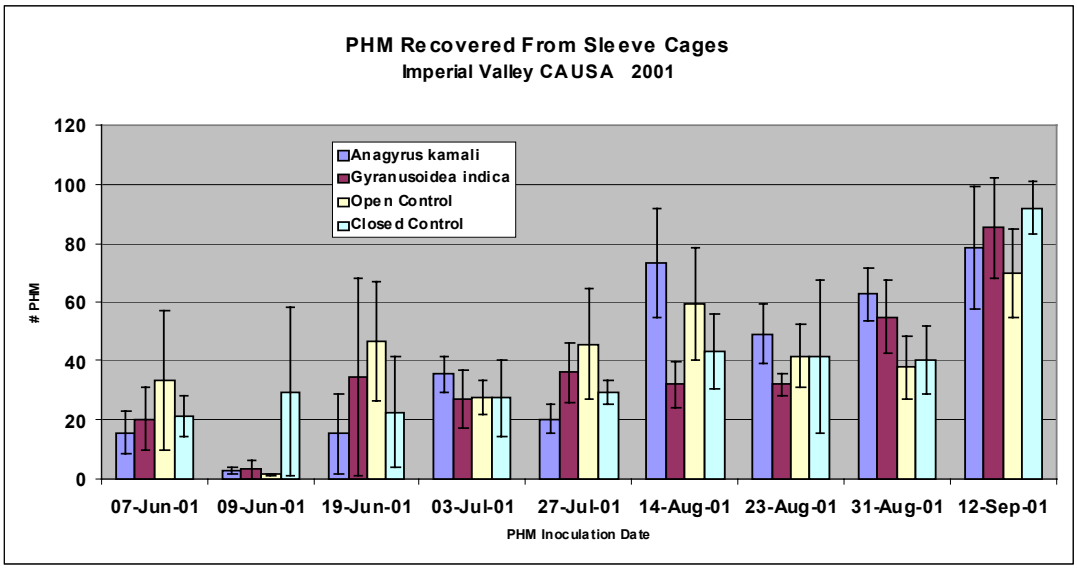
Growing terminals on Mulberry trees were inoculated with PHM crawlers, and covered with organdy sleeve cages. Subsamples were taken to determine the number of crawlers placed on each terminal. When third instar PHM nymphs were observed on the terminals, parasitoids (5 females with 5 males) were released into the cages. Between 10 and 21 days after introduction of the parasitoids, PHM were removed, isolated in gel-caps, and observed for emergence of adult parasitoids. There were four treatments: two controls [one consisting of a closed cage with PHM and no parasitoids, the other was a cage open at one end with PHM and no parasitoids] and two closed cages, one with PHM and *A. kamali* and one with PHM and *G. indica*. Treatments were set up 9 times from June through September: no inoculations were made later in the year due to the lack of suitable host plant material. The number of cages observed per treatment in each of the 9 replications varied from 2 to 5 for a total of 162 sleeve cages. Results were subjected to analysis of variance by PROC GLM in SAS Version 8 to determine if all treatments on all dates used the same number of PHM and to see if there were differences in number of parasitoids recovered and percentage parasitism among parasitoid treatments.

Results and Discussion:

There were no significant differences among treatments in the number of mealybugs recovered at the time of sample collection (Figure 1), although number of mealybugs varied with date of inoculation (Figure 2). The increasing number of PHM recovered from terminals as the year progressed is consistent with field observations. These results suggest that PHM population increase is greatly affected by an increase in survival by crawlers as ambient conditions change through the growing season. Measurement of parasitism in the later months is probably more reliable due to the higher number of available hosts at that time. In retrospect, this could be compensated for by inoculating at higher rates early in the season and lowering inoculation rate for later replications in anticipation of the shift in survival.

There were significant differences among both treatments and inoculation date in the number of parasitoids recovered (Figure 3) and in percentage parasitism (Figure 4). Fewer parasitoids were recovered from control cages than from cages in which parasitoids were released. Contamination of closed control treatment was high in the 19 June test (16%)and in the 12 September test (5.4%): each of these resulted from contamination of a single cage. Parasitism in open controls was detected from 14 August through 12 September: it was highest in the 23 August treatment (13.2%). Two *A. kamali* replicates were found to be contaminated with *G. indica* (total of 3 individuals), and two *G. indica* replicates were found to be contaminated with *A. kamali* (total of 4 individuals). Contamination levels were not sufficient to alter conclusions of this test. Both *A. kamali* and *G. indica* were active throughout the summer. The highest level of activity was in late August and September when more PHM were parasitized per female parasitoid released. The higher functional response during the late summer indicated by these tests coupled with a general increase in parasitoid numbers through the summer would allow the established parasitoid population to have a great impact on PHM populations. These results suggest that *A. kamali* and *G. indica* are equally well suited for establishment under conditions found in the Imperial Valley in contrast to field observations showing *A. kamali* to be much more common than *G. indica.* The reason for this apparent difference in performance by *G. indica* in sleeve cages versus the open environment is currently unknown.

Figure 1. Mean of numbers of potential host individuals per treatment per set of cages that were recovered and encapsulated at the time of sampling.



Bars indicate standard errors.
Significance level among treatments F=0.71, Pr>F=0.5500.
Significance level among dates F=7.12, Pr>F 0.0001

Figure 2. Mean numbers of crawlers introduced onto terminals per inoculation date (n=5). Percentage survival calculated from mean crawlers and mean number of potential host individuals recovered from all treatments in a set of cages.

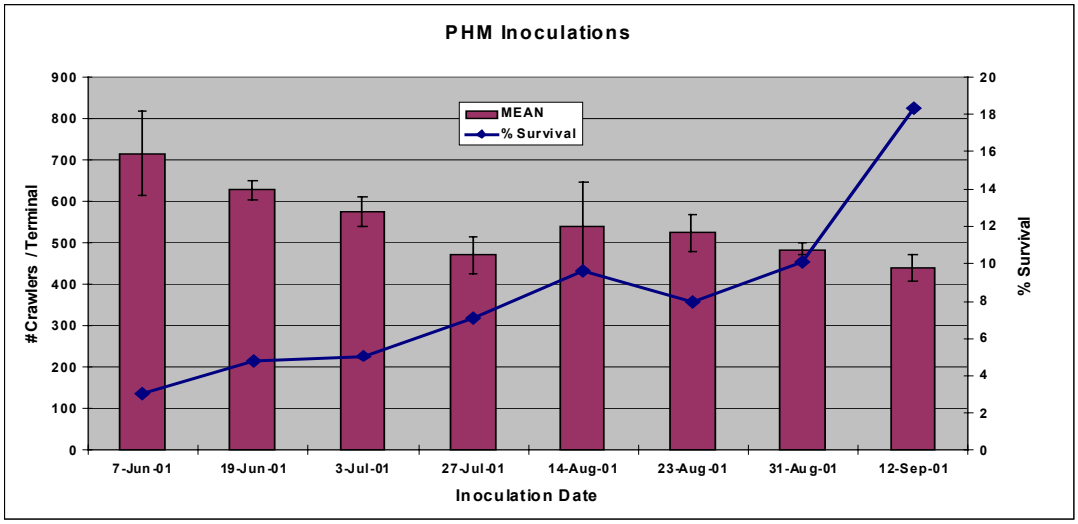
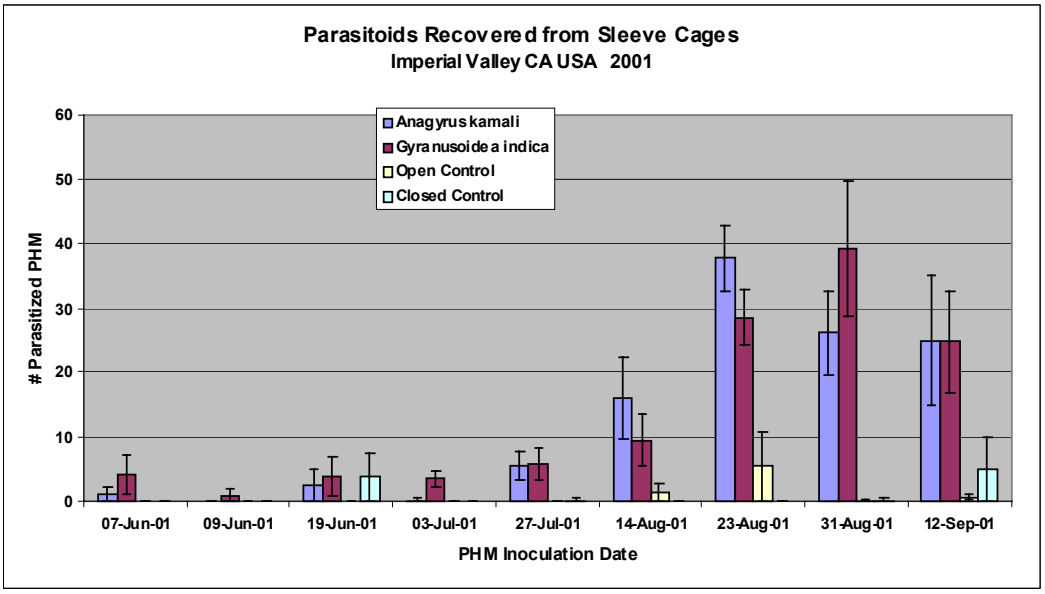
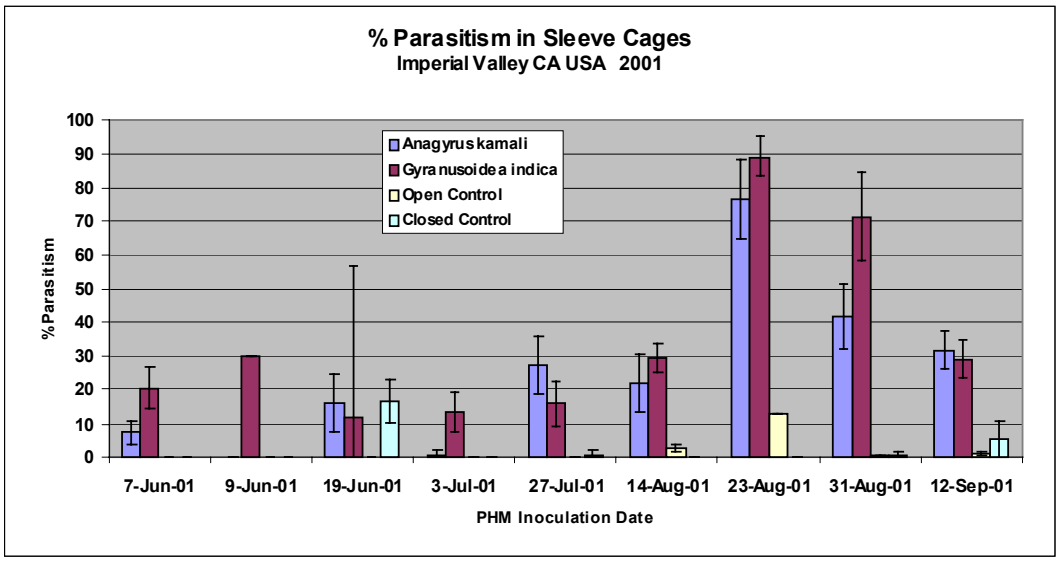


Figure 3 Mean of parasitoids recovered from each treatment in each set of cages



Bars indicate standard errors.
Significance level among treatments (F=16.97) Pr>F=0.0001
Significance level among inoculation dates (F=7.19) Pr>F=0.0001

Figure 4. Percentage of potential hosts from which parasitoids were recovered



Bars indicate standard errors.
Significance level among treatments (F=30.74) Pr>F=0.0001
Significance level among inoculation dates (F=7.05) Pr>F=0.0001

Biological Control Project in California
Section III a:
Exploration for Biological Control Agents of Pink Hibiscus Mealybug in Australia

Prepared by:
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c/o CSIRO Entomology – Long Pocket Laboratories, 120 Meiers Rd. Indooroopilly,
Queensland, Australia 4068

The Pink Hibiscus Mealybug (PHM), *Maconellicoccus hirsutus* (Green) (Homoptera: Pseudococcidae) has recently entered California and poses a serious threat to the forestry, agricultural, horticultural and tourist industries of the southern USA. It attacks 215 genera of economically useful plants worldwide. Chemical control is ineffective and Encyrtidae (Hymenoptera) parasitoids have been successfully used in biological control of PHM elsewhere. The Australian Biological Control Laboratory, in cooperation with the ARS European Biological Control Laboratory continued foreign exploration for PHM natural enemies in Western Australia. Several parasitoid and predator species have been discovered (Table 1).

Table 1. Natural enemies recovered from Pink Hibiscus Mealybug in Australia.

Species	Accession #	Location	Date	Comments
Gyranusoidea indica Hym: Encyrtidae	2000809	Brisbane, QLD	28-II-2000	D2 sequences identical to colony in Brawly, CA
Cacoxenus perspicax Diptera: Drosophilidae	2000809	Brisbane, QLD	28-II-2000	Common predator of high density PHM
Ophelosia bifasciata Hym: Pteromalidae	2000803	Brisbane, QLD	28-II-2000	Not commonly recovered may be parasitoid of *Crytpolaemus*
Crytpolaemus montrouzierri Coleoptera: Coccinellidae	2000809	Brisbane, QLD	28-II-2000	Very common predator
Encyrtidae: Hymenoptera *Coccidoctonus* sp.?	2000892	Kununurra, WA	8-X-2000	Collected from wild Malvaceous host plant, may be a hyperparasitoid
Coccophagus sp. Hymenoptera: Aphelinidae	2000892	Kununurra, WA	8-X-2000	Collected from wild Malvaceous host plant
Mataeomera sp. Lepidoptera: Noctuidae	2000892	Kununurra, WA	8-X-2000	Collected from wild Malvaceous host plant

Field Studies. Little is known about the biology of PHM in its native range where it is not a pest. Studies in its native range may be useful as a benchmark for biological control programs where mealybug is an exotic pest. With this in mind, we set-up field studies in the Brisbane area to record the seasonal phenology of PHM and its associated natural enemies. Six sites with *Hibiscus rosa-sinensis* were chosen in Sherwood, a suburb of Brisbane, for the study. Cardboard bands (6) were placed on the limbs of the hibiscus plants and collected monthly. The numbers of mealybugs and emerged parasitoids were recorded. This technique has been used by numerous mealybug researchers, and provides a standard measure of density across field sites.

Population levels of PHM peaked in the fall of 2000 and then stayed at extremely low levels through October 2001 (Fig 1). We expected to see an increase in PHM during the fall of 2001 (Feb-May), but drought conditions may have had an influence. However, even at sustained low densities, we commonly collected the predator, *Cryptolaemus montrouzieri*. This suggests that natural enemies play an important role in regulating PHM populations in its native range. We will continue the study through 2002.

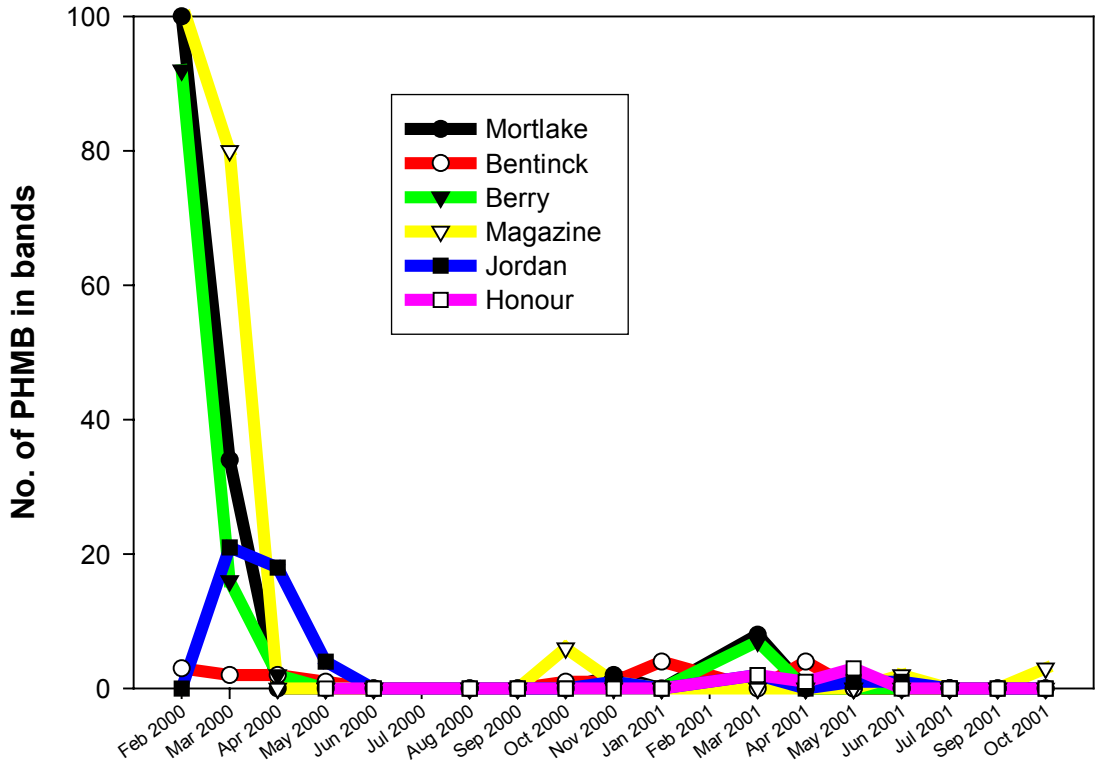

Fig 1. Seasonal population levels of PHM at six locations in Queensland, Australia.

Biological Control Project in California
Section III b:
Biological Control Agents of Pink Hibiscus Mealybug in Egypt

Based on communication with Dr. Dan Gonzalez, Univ. Calif. Riverside

Working in cooperation with Dr. Ahmed El-Heneidy, Dept. of Biological Control, PPI, Ministry of Agric., Ciro Egypt, Dr. D. Gonzalez, University of California, Riverside traveled to Egypt during November of 2001 to again collect parasitoids in the arid southern region of the country. *Anagyrus kamali* was again collected and sent to the USDA-APHIS quarantine facility in Mission, Texas. This population was processed through quarantine and received by CDFA at the El Centro insectary in January 2002. This population is being reared for release in 2002. Whereas the culture of *A. kamali* released from 1999 to 2001 was a mixed culture originating from China, Hawaii and Australia, the present population comes from an area representing a better climatic match to the climate in the United State's, desert southwest region.

Development of New Pesticide Options

Efficacy of Systemic Neonicotinoid Insecticides Against Pink Hibiscus Mealybug On Trees and Shrubs in Imperial Valley

Prepared by:
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[2]Department of Entomology, University of California, Riverside

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green), was first discovered in the Imperial Valley, CA in August, 1999. A survey conducted soon after its discovery determined that large areas at the southern end of the valley, including sites along the US-Mexico border in Mexicali, B.C., were infested with PHM. The survey results provided confirmation that PHM was indeed established in the Imperial Valley, thus representing the first recorded infestation on the North American mainland. Previously, PHM had been discovered on the island of Grenada in the Caribbean in 1994, followed by discoveries on more than 25 islands the next few years. The series of PHM discoveries on islands across the Caribbean basin was largely interpreted as rapid colonization by a pest with high dispersal capability. Hence, many supposed that establishment on the North America mainland would result in rapid spread between locales.

The infestation zone in the Imperial Valley has in fact remained relatively static in the 2+ years since PHM was detected. Movement from one site to the next, or between plants within a site, appears to be very slow. Dispersal is probably dependent principally upon the wind, and first instar crawlers may be an important dispersal stage given their small size and mobility. Additional sites within the Imperial Valley will undoubtedly be discovered, as incipient infestations already established become apparent as they develop. However, the importation and establishment of parasitoids against PHM has no doubt been critical in reducing infestation densities and hence the amount of dispersal between sites.

A management program that emphasizes containment and suppression of PHM within its current infestation zone will require tools capable of eliminating new infestation sites as they are discovered. A new class of systemic insecticides, known as neonicotinoids, provides a safe and effective means of eliminating whole plant infestations with just a single soil-applied treatment. The following report presents results obtained thus far with 2 neonicotinoids, imidacloprid and thiamethoxam, against PHM.

Methods

Trees and shrubs were treated with either thiamethoxam or imidacloprid by injecting the formulated materials into the root zone of each plant. The top-label rate for each material (thiamethoxam = Meridian® = 4 g/inch DBH; imidacloprid = Merit® = 1.4 tsp/inch DBH) was mixed with 4 l of water inside the reservoir of the Kyoritsu hand soil-injector. The mixture was then dispensed in 5 ml aliquots with each press of the spring-loaded injector. Injections were made around the circumference of each tree or shrub and from the base of the plant to the canopy 'drip line'.

Prior to treatments, subject trees were sampled to determine the pre-treatment infestation densities. The level of infestation of each terminal was described as light, moderate or heavy based on estimated PHM densities of 1-25, 26-100, >100, respectively. Following treatment applications, treated trees or shrubs at each site were intensively sampled periodically to determine the fate of PHM infestations. Branches and terminals that showed obvious signs of PHM infestation were collected preferentially to ones without apparent symptoms of infestation. Each sampled terminal was dissected under a compound microscope and carefully inspected for live PHM. All live individuals on each terminal were counted and identified to stage.

Summary – Year 2000 Applications

- A total of 12 trees and 1 shrub were treated once with either thiamethoxam or imidacloprid. Periodic evaluations from August to November, 2000, revealed substantial reductions in infestations, and in some cases, complete elimination of infestations.
- Visual inspection of all 13 plants in late June, 2001, revealed the absence of infestations on all treated plants. However, even untreated control plants appeared to be free of infestations.
- A thorough visual inspection and sampling of branches (20 per plant) in early October, 2001, revealed a *complete absence of PHM on 10 of 13 treated plants*. On 2 of 13 plants, 1 branch out of 20 from each tree revealed the presence of PHM, but as crawlers only, thus suggesting a recent re-infestation of these 2 trees (at Camacho's). The only plant showing a carry-over infestation from the previous year was the carob tree in Holtville. Untreated mulberry trees at Camacho's showed moderate to heavy infestations.

Table 1. Summary of plants treated in 2000 and their current infestation status.

Treatment Date	Treatment	Location	Plant Type	Plant No.	Circumference (inches)	Infestation at Time of Treatment[†]	Current Infestation Status[*]
27 July	Thiamethoxam	Camacho's	mulberry tree	1	21	Heavy	Free[‡]
				2	18		
				3	16		
				4	12		
				5	25		
				6	19	moderate	
27 September	Thiamethoxam	I-8 Rest stop	mulberry tree	7	26	light	Free
		IVC	mulberry tree	8	28	light	Free
		Holtville	hibiscus shrub	1	32	moderate	Free
			carob tree	1	54	moderate	Infested
		Camacho's	mulberry tree	9	12	heavy	Free
6 October	Imidacloprid	Hashem's	mulberry tree	10	24	moderate	Free
			mulberry tree	11	23	moderate	Free

[†] Heavy, moderate or light are based on the extent that a tree or shrub volume are infested as well as the intensity of infestation throughout. 'Heavy' requires that all samples from around a tree or shrub are infested, with at least half of them having >100 PHM. 'Light' will be an incomplete infestation, with less than 10% of the samples having >100 PHM. 'Moderate' will have at least 75% infested branches, with 25% having >100 PHM.
[*] Based on visual inspections and 20 branch samples collected from each plant in early October, 2001.
[‡] A single branch tip on each of 2 trees was infested with 12-30 crawlers, probably representing a re-infestation.

Summary – Year 2001 Applications

- A total of 7 trees were treated in early October. Less than 3 weeks post-treatment, very few live PHM remain on the 3 mulberry trees treated at the I-8 rest-stop. Higher numbers of live PHM remain on the 3 mulberry trees treated in Imperial and on the silk oak tree treated in Seeley, even though these constitute lighter infestations than at the I-8 rest-stop. Differences in the rate of mortality may be due to the large size of the silk oak tree in Seeley, but also due to less accessibility to the roots of the 3 mulberry trees in Imperial (see Discussion).

Table 2. Summary of plants treated in 2001 and their current infestation status.

Treatment Date	Treatment	Location	Plant Type	Plant No.	Circumference (inches)	Infestation at Time of Treatment†	Current Infestation Status*
9 October	Thiamethoxam	I-8 Rest stop	mulberry tree	1	36	heavy	Virtually All Dead
				2	31	heavy	
		Seeley	silk oak tree	1	58	moderate	Some dead, most alive
	Imidacloprid	I-8 Rest stop	mulberry tree	3	31	moderate	Virtually All Dead
10 October	Imidacloprid	Imperial	mulberry tree	4	33	moderate	Most Dead, some alive
				5	60	light	
	Thiamethoxam	Imperial	mulberry tree	6	41	moderate	

† See footnote in Table 1 for explanation.
* Samples collected 28 Oct. 2001

Discussion

The performance of both neonicotinoid compounds proved outstanding in nearly every instance. A single application was sufficient to completely eliminate PHM infestations in all 11 mulberry trees and the lone hibiscus shrub treated in 2000. The only plant not rid of its PHM infestation was the large carob tree located in Holtville. This was the largest of the treated trees, but also the only one growing at an acute angle from perpendicular. Thus, it was less certain where the root zone would be concentrated and where the insecticide should be injected. A similar problem, i.e. uneven distribution of the insecticide throughout the root zone, was encountered in 2001 with 3 mulberry trees in the city of Imperial. All 3 trees were growing at the edge of a homeowner's front yard that was bordered by the dirt shoulder of the frontage road. The compaction of the dirt shoulder made it impossible to inject insecticide into ½ of the root zone, i.e. the roadside semicircle. The opposite semicircle on the yard side was planted in grass and was the only area that the Kyoritsu injector could be inserted. Consequently, the activity against PHM was noticeably delayed relative to 3 mulberry trees at the I-8 rest stop treated 1 day earlier. At the time of evaluation 18-19 days post-treatment, virtually all PHM collected from the I-8 mulberry trees were dead compared to the Imperial mulberry trees where many PHM were still alive. Follow-up evaluations of all 6 trees in 2002 will tell if the limited access to the root zones of the Imperial mulberry trees precluded the complete elimination of PHM.

Despite the occasional difficulties in applying materials to some trees, the neonicotinoids show great potential as effective tools for containing and suppressing PHM within its current zone of infestation in the Imperial Valley. The combination of biocontrol and treatment of peripheral infestations with neonicotinoids should be effective at reducing the area infested by PHM in the Imperial Valley. See appendix D for detailed records.

Mealybug Host Plant Preference Study

Host Preference and Suitability of the Pink Hibiscus Mealybug, *Macconelliccocus hirsutus*

Dr.Timothy Paine, Chris Hanlon, and Sarah Allen
Department of Entomology
University of California, Riverside

The pink hibiscus mealybug, a recent addition to California's fauna, is currently restricted to an area in Imperial County in and around El Centro. The wide host range exhibited by this insect pest in other parts of the world are a cause for concern in a state with such an extensive variety of plant species, comprising native and non-native, ornamental, and agricultural plants. The plants chosen for this test reflect some of this diversity.

Plant	Native?	Type
Live Oak	Yes	Ornamental
Sycamore	Yes	Ornamental
Baccharis	Yes	Ornamental
Hibiscus	No	Ornamental
Grape	No	Agricultural
Lemon	No	Agricultural

Experimental Design:

I. Host preference determined by choice test.
   1. Six plants of different species were placed in a 9 sq. ft. block in random order.
   2. Crawlers were distributed evenly over each species, using a central dispersal plate.
   3. Host preference was measured by counting the number of adults established on each plant after 3 weeks.

II. Host suitability determined by no choice test.
   1. Six plants of the same species were placed in a 9 sq. ft. block in random order.
   2. Crawlers were distributed evenly over each block, using a central dispersal plate made from PVC and a plastic plate.
   3. Host suitability was determined after 7 weeks by counting:
      A. the number of established individuals
      B. the number of egg masses per plant

Results and discussion:

In the "no-choice" experiment, the PHM survived equally well on all plants except the live oak. When counting the number of egg masses, the hibiscus was the most favorable to mealybug reproduction (Tables 1 and 2). When the crawlers were given a choice amongst these six plant species, hibiscus was overwhelmingly preferred, especially if percentage of PHM plant choice is examined (Tables 3 and 4).

Several months after sampling the plants in the "choice" experiment, we resampled, taking four 30cm branch tips from each plant and counted the mealybugs and eggmasses. Again, hibiscus was overwhelmingly the preferred host (Tables 5 and 6).

While it was not surprising to find hibiscus as the favored plant, it was surprising to see the amount of variability in the responses to some of the other plants. In the "no-choice" experiment, PHM performed very well on grape. However, this plant was completely avoided when the insects were given other options. Lemon was highly placed on each comparison table except when number of egg masses per plant was considered. Sycamore and Baccharis were only moderately attractive and supportive, while live oak was the least favored by PHM overall.

Table 1. PHM "no-choice" experiment.
Mean number of PHM per plant

Plant	Mean(SE)	SNK Group
Grape	14.40(4.37)	A
Lemon	10.83(3.53)	A
Sycamore	9.50(2.74)	A B
Hibiscus	7.17(1.82)	A B
Baccharis	4.50(0.33)	A B
Live Oak	0.00(0.00)	B

F=3.77 Pr>F=0.0095

Table 2. PHM "no-choice" experiment.
Mean number of egg masses per plant.

Plant	Mean(SE)	SNK Group
Hibiscus	5.33(1.65)	A
Grape	1.20(0.97)	B
Sycamore	0.83(0.48)	B
Baccharis	0.33(0.33)	B
Lemon	0.00(0.00)	B
Live Oak	0.00(0.00)	B

F=6.56 Pr>F=0.0003

Table 3. PHM "choice" experiment.
Mean number of PHM per plant.

Plant	Mean(SE)	SNK Group
Hibiscus	18.50(7.91)	A
Lemon	2.67(1.09)	B
Sycamore	1.50(0.81)	B
Baccharis	0.83(0.65)	B
Grape	0.00(0.00)	B
Live Oak	0.00(0.00)	B

F=4.82 Pr>F=0.0024

Table 4. PHM "choice" experiment
Percentage of PHM plant choice

Plant	Mean pct.(SE)	SNK Group
Hibiscus	66.55(11.54)	A
Lemon	15.56(10.38)	B
Baccharis	12.63(10.91)	B
Sycamore	5.26(2.98)	B
Grape	0.00(0.00)	B
Live Oak	0.00(0.00)	B

F=11.48 Pr>F=<0.0001

Table 5. PHM "choice" experiment, second sample.
Mean number of PHM per sample (30cm branch tip)

Plant	Mean(SE)	SNK Group
Hibiscus	26.83(5.92)	A
Lemon	1.54(0.49)	B
Baccharis	0.91(0.31)	B
Grape	0.88(0.57)	B
Sycamore	0.83(0.28)	B
Oak	0.75(0.36)	B

F=16.44 Pr>F <0.0001

Table 6. PHM "choice" experiment, second sample.
Mean number of eggmasses per sample (30cm branch tip)

Plant	Mean(SE)	SNK Group
Hibiscus	16.58(3.89)	A
Oak	0.55(0.32)	B
Grape	0.19(0.14)	B
Baccharis	0.18(0.10)	B
Sycamore	0.08(0.08)	B
Lemon	0.00(0.00)	B

F=15.53 Pr>F <0.0001

Status of Pink Hibiscus Mealybug in Northern Mexico

Based on information presented by Ing. Cesar Cota Gomez, at the November 2001 meeting in El Centro, California

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The PHM was detected at sites in Mexicali, along the border of Mexico and the United States during September of 1999. To date, the PHM distribution is limited to the Mexicali Valley. It is predominantly located within several miles south of the border.

Coinciding with the release of two parasitoid species (*Anagyrus kamali* and *Gyranusoidea indica*) in Imperial Valley, California, during September of 1999, several thousand parasitoids were released by USDA-APHIS (under the direction of Dr. D. Meyerdirk) at several infested sites in Mexicali, Mexico in cooperation with Mexican officials.

In 1999 and 2000, Mexican authorities were required to eradicate the pest. However, during 2001 there was greater emphasis on using classical biological control to manage the pest. During 2001, *A. kamali* and *G. indica* were provided to Mexico and released throughout the infested area. Over 100,000 parasitoids produced at the CDFA insectary in El Centro were provided.

Acknowledgements

Thanks are given to Ted Boratynski (USDA-APHIS) and Eduardo Gutierrez (USDA-APHIS-IS) for their considerable cooperation in facilitating the delivery of parasitoids.

APPENDICES

Appendix A – Western hemisphere distribution of PHM

Appendix B – Imperial Valley survey plan and data maps

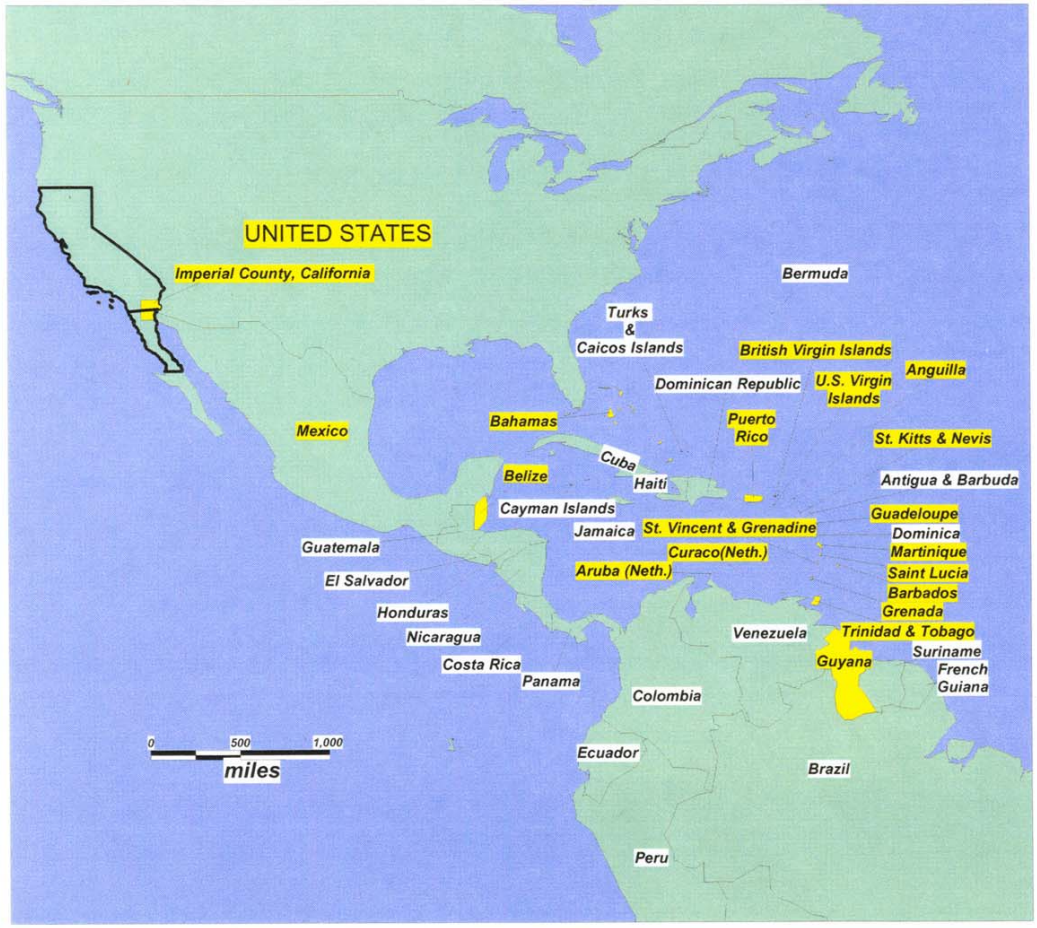
Appendix C – Biological control evaluation graphs

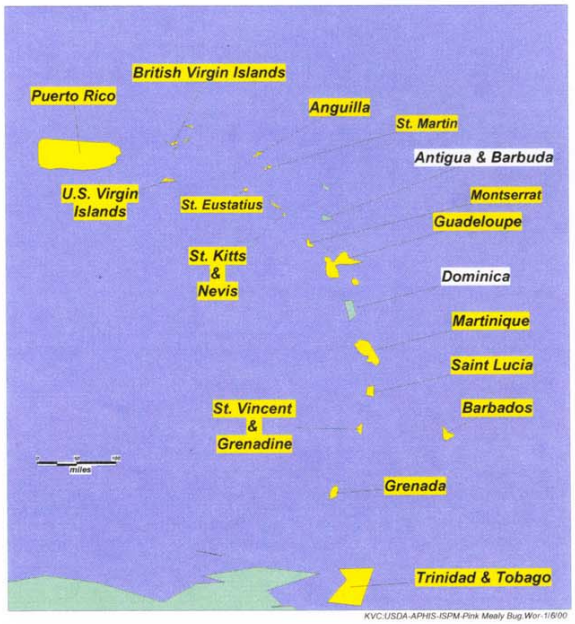
Appendix D – Insecticide evaluation records

Appendix E – Cooperative Program for the Control of Pink Hibiscus Mealybug in Imperial County

Pink Hibiscus Mealybug, *Maconellicoccus hirsutus (Green)* Western Hemisphere Distribution

(as of January 4, 2001)

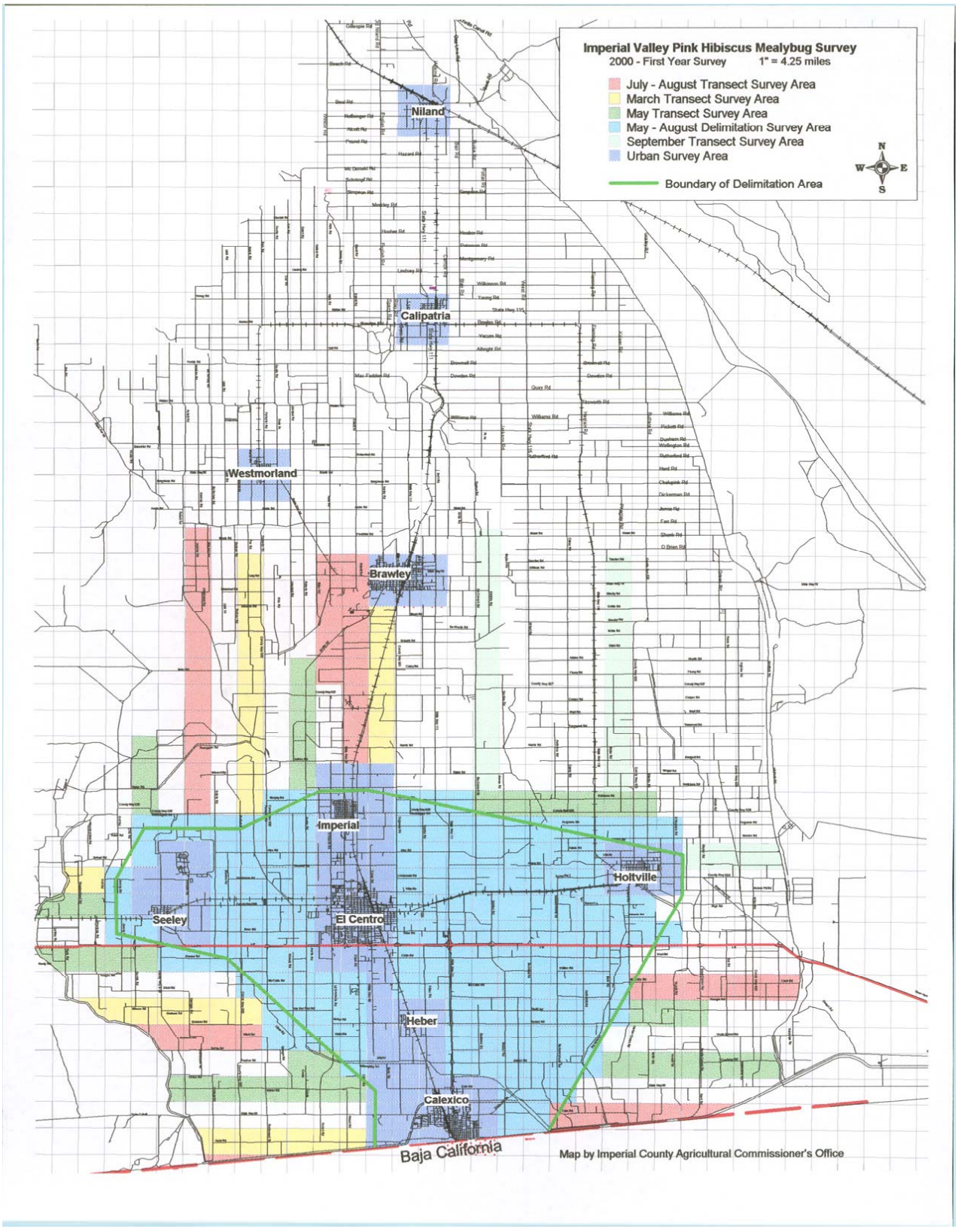




USDA, Animal and Plant Health Inspection Service

Appendix A

APPENDIX B1


Imperial Valley Pink Hibiscus Mealybug Survey
2000 - First Year Survey 1" = 4.25 miles
July - August Transect Survey Area
March Transect Survey Area
May Transect Survey Area
May - August Delimitation Survey Area
September Transect Survey Area
Urban Survey Area
Boundary of Delimitation Area
N
W
E
S
Niland
Calipatria
Westmorland
Brawley
Imperial
Holtville
Seeley
El Centro
Heber
Calexico
Baja California
Map by Imperial County Agricultural Commissioner's Office

2000 Pink Hibiscus Mealybug Infestation, Imperial County.

Data from March-Nov., 2000. Showing points geocoded as of 23 Jan. 2001.

APPENDIX B2

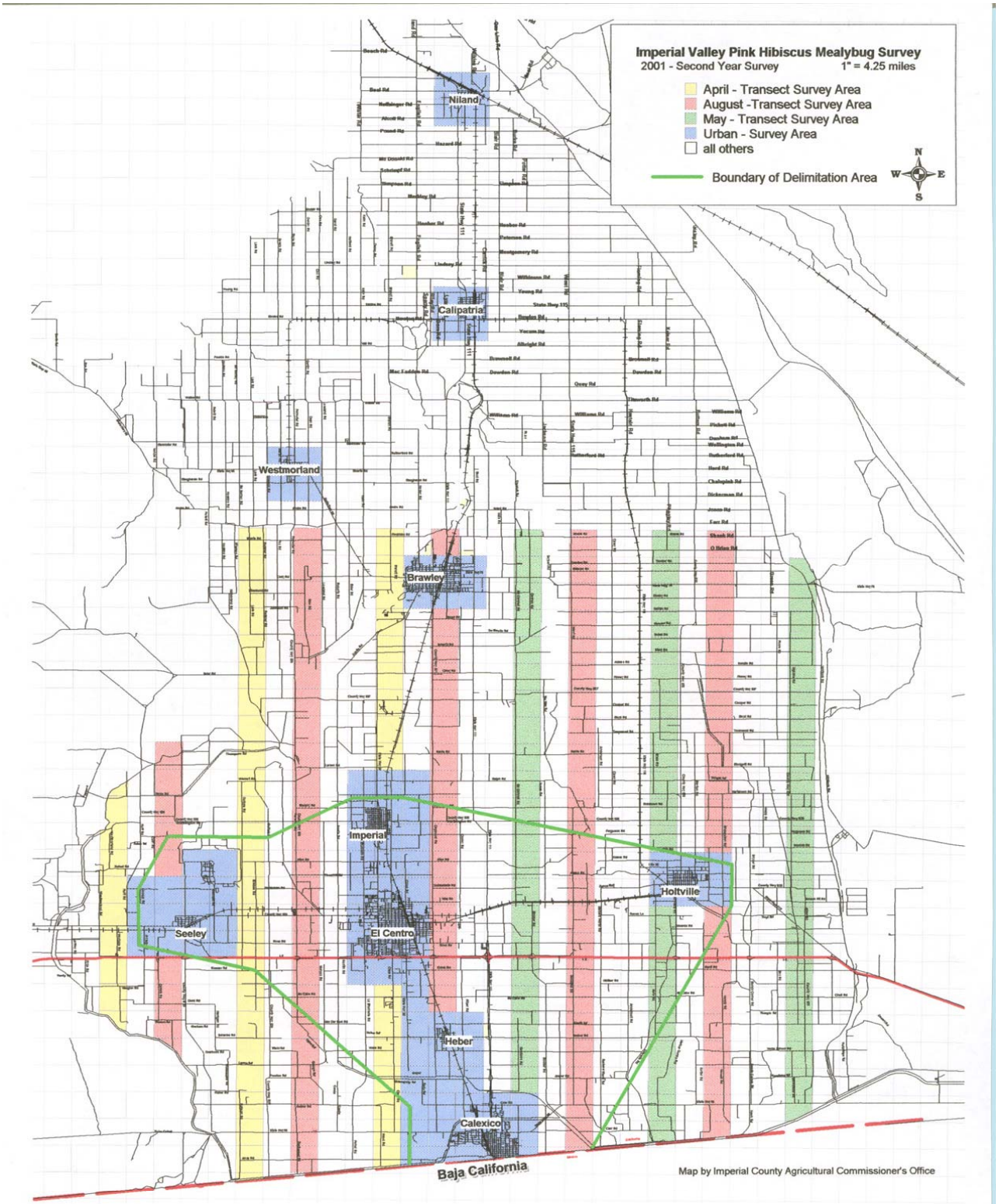
Niland
Calipatria
Westmorland
Brawley
Imperial
El Centro
Holtville
Seeley
Heber
Calexico
Salton Sea
US Highway 111
State Hwy 86
County Hwy S30
Rutherford Rd
Forrester Rd
State Hwy 86
State Hwy 111
State Hwy 115
County Hwy S27
Highline Rd
County Hwy S28
I-8
State Hwy 98
Yuha Cutoff
0 2.5 5
Miles

■ = Infested Sites (794 plottable of 849)

● = Uninfested Sites (13,830 plottable of 14,725)

Data and Map by CDFA, Integrated Pest Control Branch, 1/22/01
V:\Maps\PinkHibiscus Mealybug\Geocoded23Jan01\PHM23Jan01FullArea.mxd 45-8x11.WOR

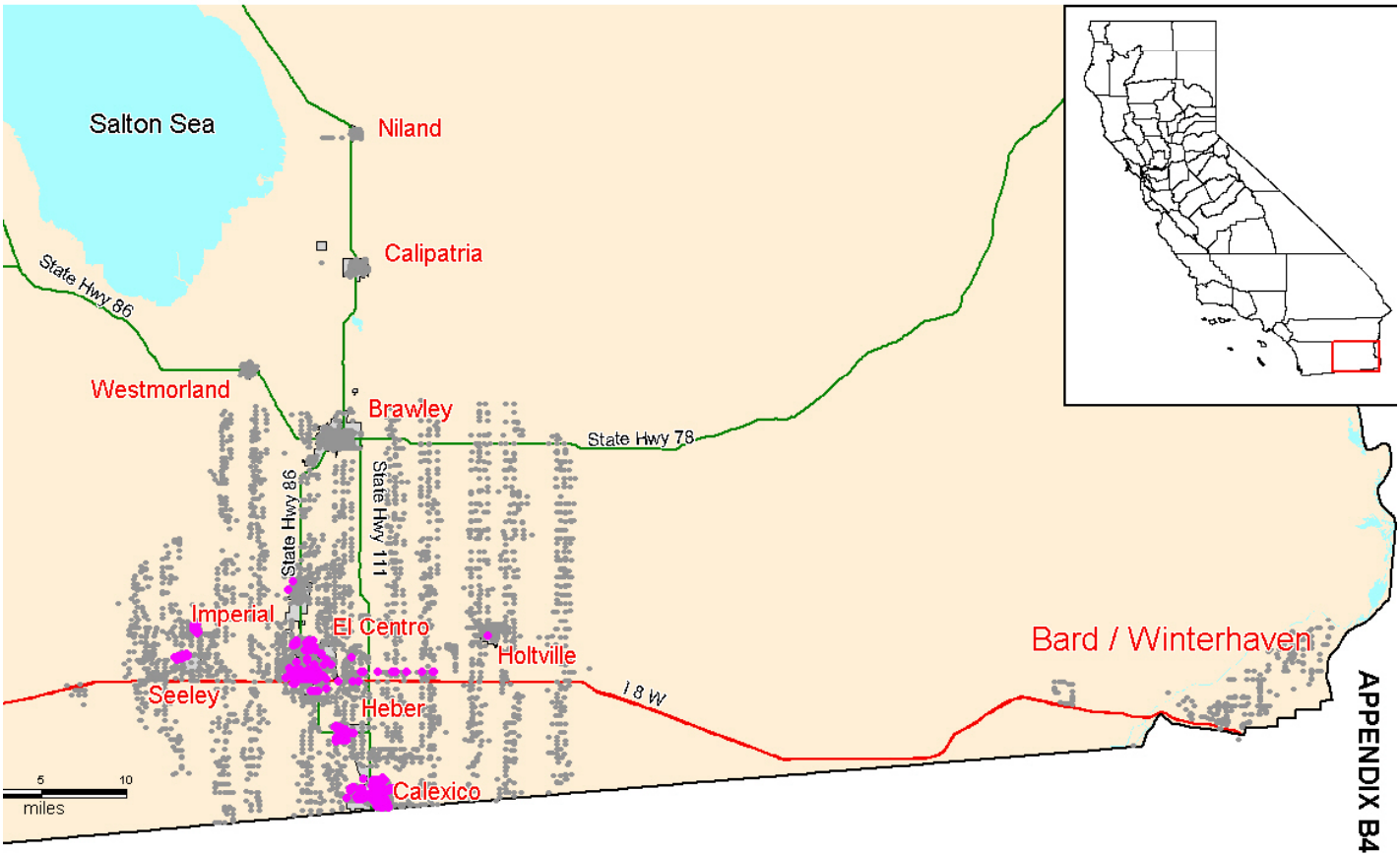
APPENDIX B3


Imperial Valley Pink Hibiscus Mealybug Survey
2001 - Second Year Survey
1" = 4.25 miles
April - Transect Survey Area
August -Transect Survey Area
May - Transect Survey Area
Urban - Survey Area
all others
Boundary of Delimitation Area
N
W
E
S
Niland
Calipatria
Westmorland
Brawley
Imperial
Holtville
Seeley
El Centro
Heber
Calexico
Baja California
Map by Imperial County Agricultural Commissioner's Office

APPENDIX B4 : IMPERIAL VALLEY

2001 Pink Hibiscus Mealybug Infestation, Imperial County.

Data from April-Sept., 2001. Showing points geocoded as of 24 Jan. 2002

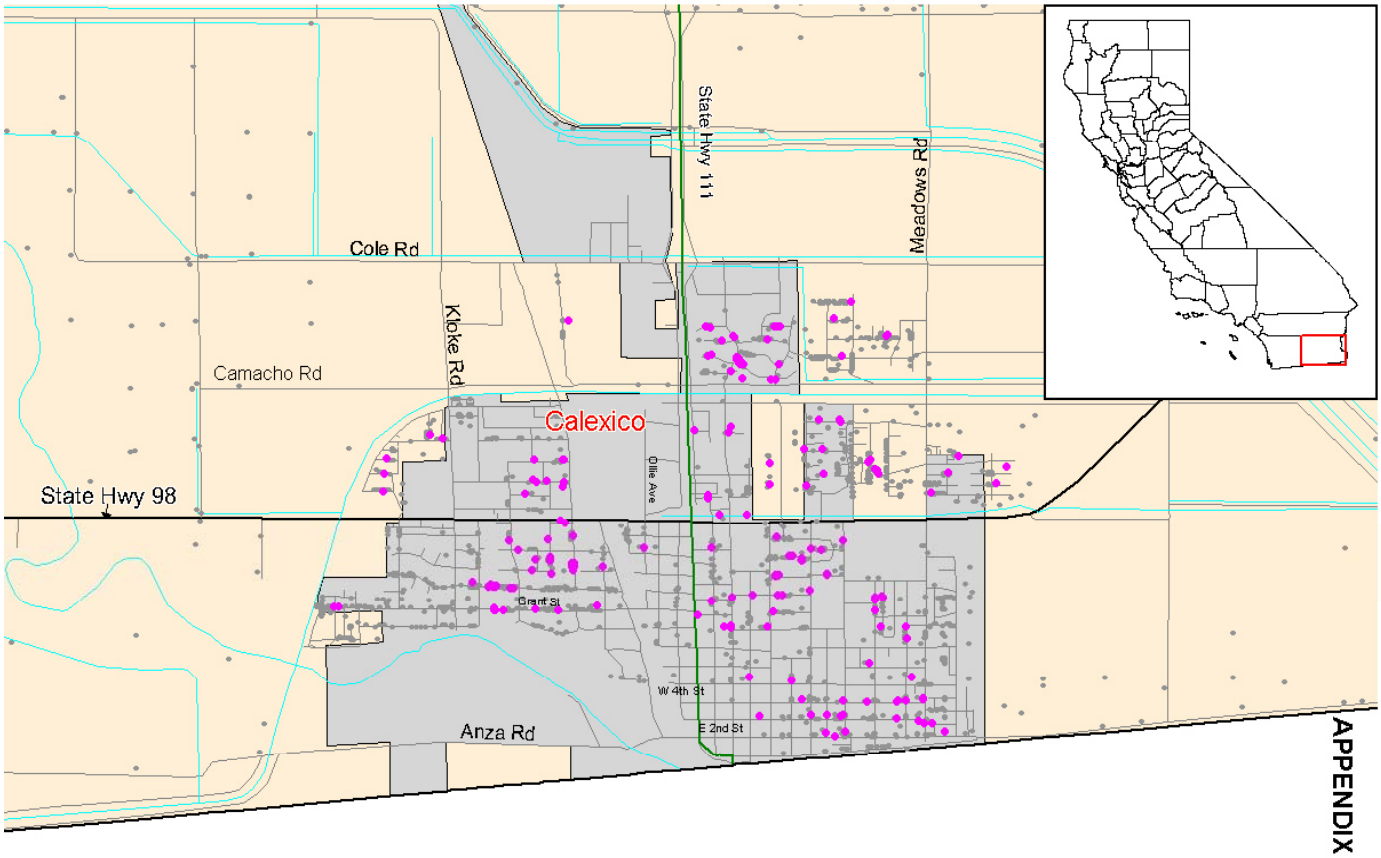


APPENDIX B4

APPENDIX B5 : CITY OF CALEXICO

2001 Pink Hibiscus Mealybug Infestation, Imperial County.

Data from April-Sept., 2001. Showing points geocoded as of 24 Jan. 2002

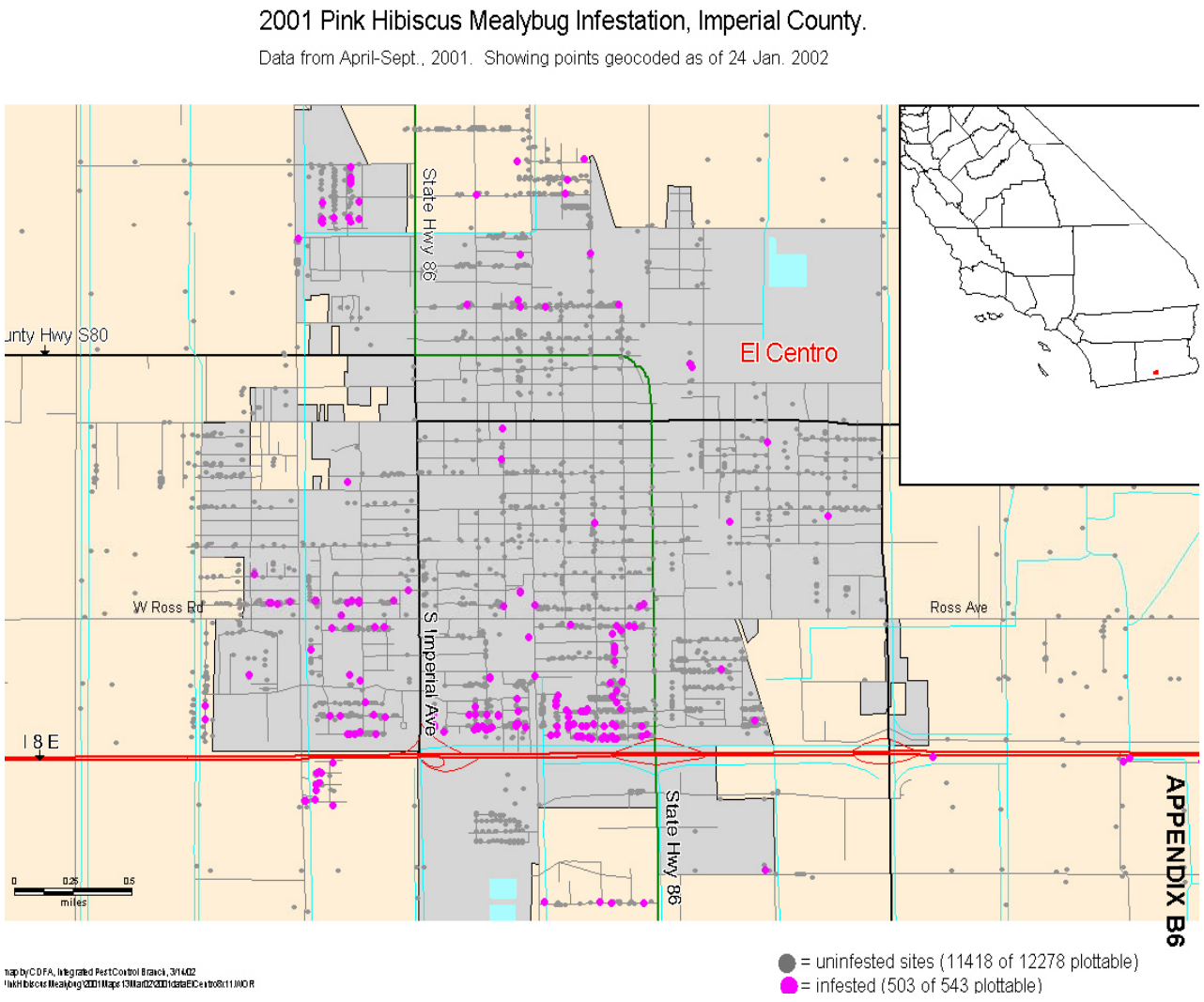


Data and map by C.D.F.A., Integrated Pest Control Branch, 3/14/02
V:\Maps\PinkHibiscusMealybug\2001\Maps13Mar02\2001dataCalexico8x11JWDR

● = uninfested sites (11418 of 12278 plottable)
● = infested (503 of 543 plottable)

APPENDIX B5

APPENDIX B6: CITY OF EL CENTRO

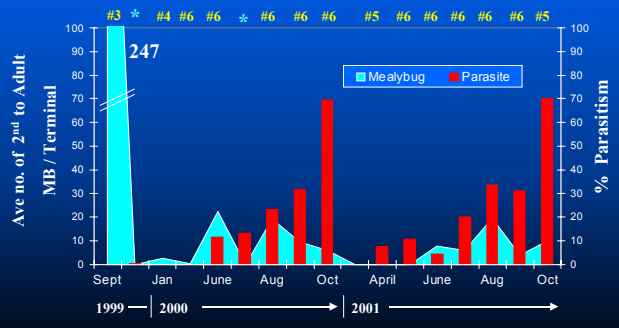


APPENDIX C1

PINK HIBISCUS MEALYBUG BIOLOGICAL CONTROL
OVERVIEW

MEAN POPULATION AND ANNUAL TEMPERATURE PATTERNS IN IMPERIAL VALLEY, CALIFORNIA (FALL 1999 - 2001)

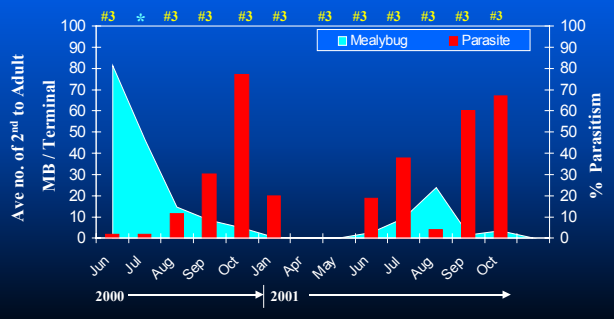
Pink Hibiscus Mealybug on Mulberry C2
Imperial Valley, California



* PHM not sampled, parasitism accessed

Note: # = Sample Size (i.e., no. of sites)

Pink Hibiscus Mealybug on Carob Tree C3
Imperial County, California

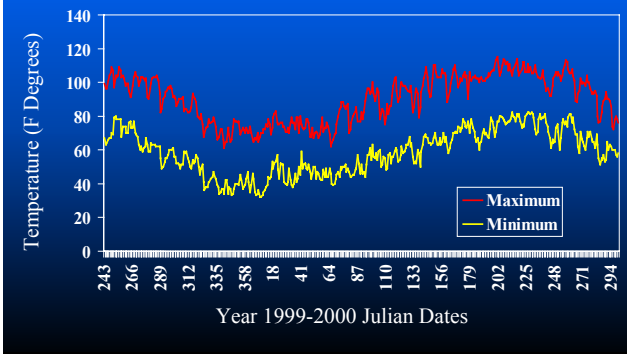


* PHM not sampled

Note: # = Sample Size (i.e., no. of sites)

C4

Minimum and Maximum Daily Temperatures Imperial Valley, California (Meloland)

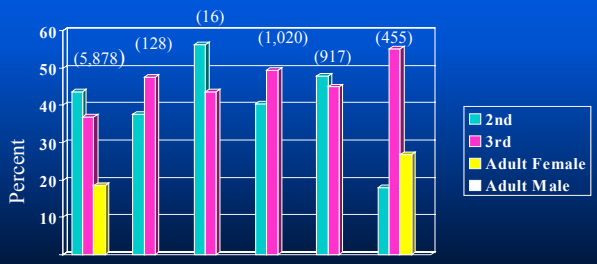


High and Low Temperature Characteristics of Imperial Valley, California (Meloland) C5

(Sept. 1999 through Sept. 2000)

Temperatures (F Degrees)	Number of Days (Max. or Min. Temp.)
100-104	76
105-109	46
110 & above	21
32 or less	2
33-39	35

Seasonal Abundance of Pink Hibiscus Mealybug Life Stages– Imperial Valley, California (Mulberry) C6

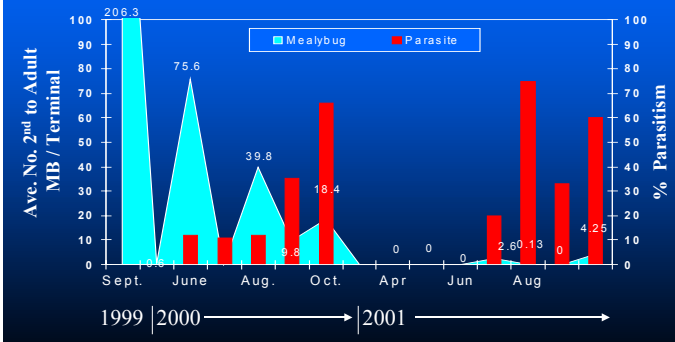


C7

MEALYBUG DENSITIES AND PARASITISM PATTERNS BY SITE AND HOST PLANT SPECIES [MULBERRY, HIBISCUS & CAROB]

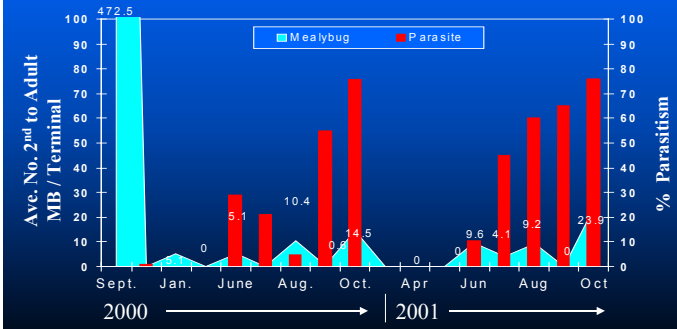
Pink Hibiscus Mealybug in Imperial Valley, California – Eady Ave, Calexico (Mulberry)

C8



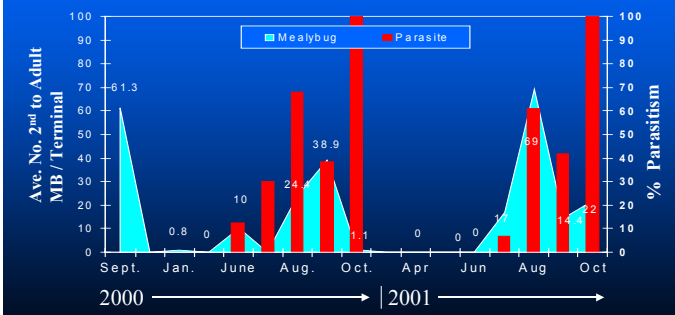
Pink Hibiscus Mealybug in Imperial Valley California – Sandalwood Dr., El Centro (Mulberry)

C9



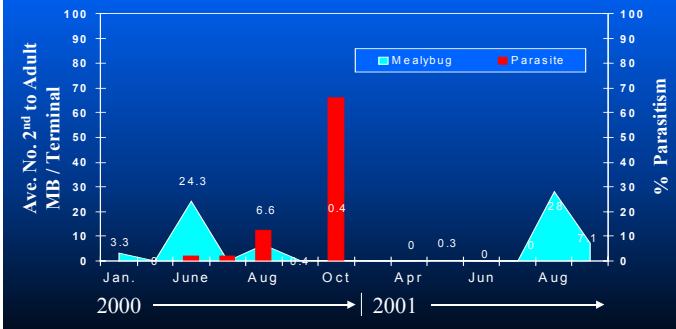
Pink Hibiscus Mealybug in Imperial Valley, California – La Brucherie Rd., El Centro (Mulberry)

C10



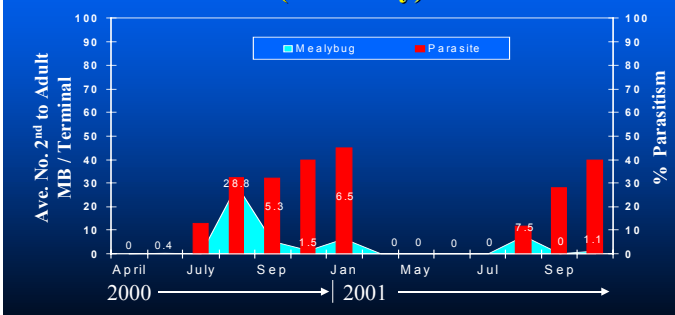
Pink Hibiscus Mealybug in Imperial Valley, California – Cole Rd., Calexico (Mulberry)

C11



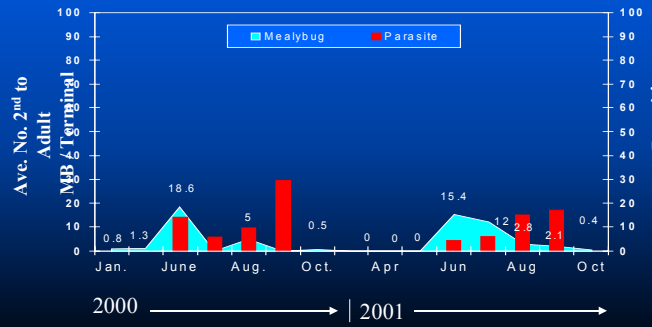
Pink Hibiscus Mealybug in Imperial Valley, California – Santa Rosa, El Centro (Mulberry)

C12



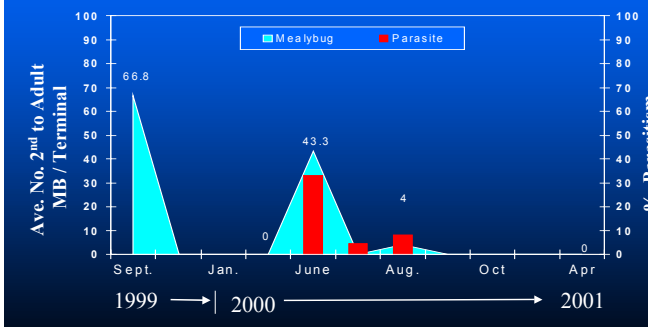
Pink Hibiscus Mealybug in Imperial Valley, California – Lincoln St.,Calexico (Mulberry)

C13



Pink Hibiscus Mealybug in Imperial Valley, California – Cole Rd. Calexico (Hibiscus)

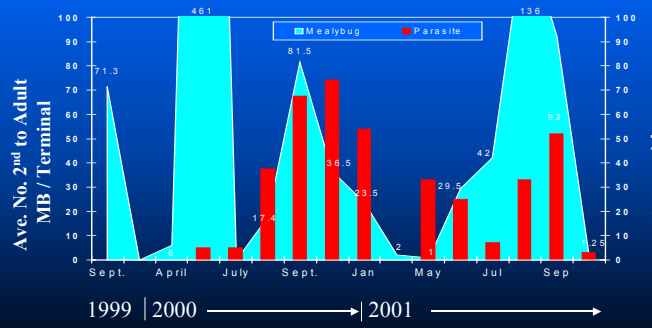
C14



Last sample: April 2001

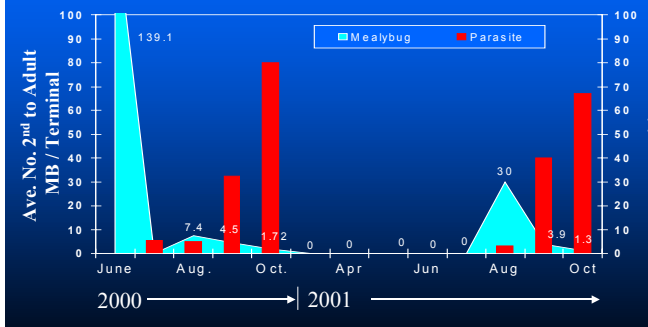
Pink Hibiscus Mealy bug in Imperial Valley, California – La Brucherie Rd., El Centro (Hibiscus)

C15



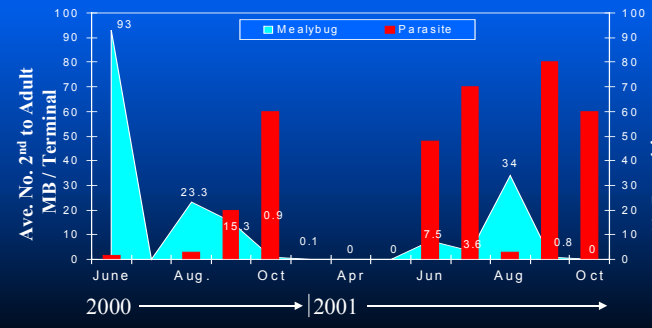
Pink Hibiscus Mealybug in Imperial Valley, California – Giles Ave., Calexico (Carob)

C16



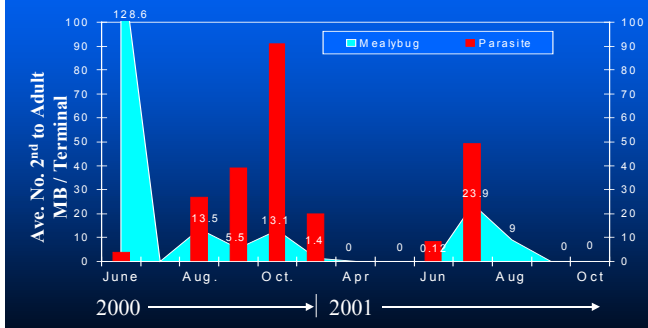
Pink Hibiscus Mealybug in Imperial Valley, California – First St., Calexico (Carob)

C17



Pink Hibiscus Mealybug in Imperial Valley, California – Sixth St., Calexico (Carob)

C18



Only 1 mealybug for Aug 01 sample

Appendix D1.

First numerical evaluation of hibiscus mealybug-infested mulberry trees at Camacho's Place Restaurant in El Centro, CA following treatment with thiamethoxam systemic insecticide on 28 July 2000. Infestation categories were based on the total number of live and dead hibiscus mealybugs in each branch terminal. Infestation rates (in summary rows) are composed of the number of terminals that had even 1 live mealybug present (numerator) relative to the total number of branches sampled (denominator).

Sample Date	Days Post-treatment	Tree No.	Branch Terminal	Infestation Category			Number Alive		
				1-25	26-100	>100	Crawlers	Nymphs	Adults
Aug. 24	28	1	1			●	0	4	0
			2			●	0	0	0
			3			●	14	4	3
			4			●	many	many	6
		2	1			●	0	0	0
			2			●	0	2	0
			3			●	0	0	0
			4			●	0	0	0
		6	1			●	0	0	0
			2			●	0	0	0
			3			●	0	7	0
			4			●	0	0	0
		7	1			●	0	0	0
			2			●	0	0	0
			3			●	0	0	0
			4			●	0	0	0
Aug. 24	Summary	Trees	Terminals				Infestation Rate		
		4	16			16	2/16	5/16	2/16

Sample Date	Days Post-treatment	Tree No.	Branch Terminal	Infestation Category			Number Alive		
				1-25	26-100	>100	Crawlers	Nymphs	Adults
Aug. 31	35	1	1			●	0	0	0
			2			●	0	0	0
			3			●	0	0	0
			4			●	0	0	0
			5			●	0	0	0
			6			●	0	0	0
		2	1			●	0	5	4
			2			●	0	0	0
			3			●	0	9	5
			4			●	0	4	2
		3	1			●	0	0	0
			2			●	0	0	0
			3			●	0	0	0
			4			●	0	0	0

			5			●	0	0	0
Aug. 31	35	4*	1			●	Untreated—All live stages		
			2			●	Untreated—All live stages		
		5	1			●	0	0	0
			2			●	0	0	0
			3			●	0	1	0
		6	1			●	0	0	0
			2			●	0	0	0
			3			●	12	6	2
			4			●	0	4	0
			5			●	22	4	2
			6			●	0	0	0
		7	1			●	15	5	1
			2			●	0	3	0
			3			●	0	0	0
Aug. 31	Summary	Trees	Terminals				Infestation Rate		
		6	27	0	0	27	3/27	9/27	6/27

* Untreated mulberry tree

Sample Date	Days Post-treatment	Tree No.	Branch Terminal	Infestation Category			Number Alive		
				1-25	26-100	>100	Crawlers	Nymphs	Adults
Sep. 7	42	1	1			●	0	0	0
			2			●	0	2	0
			3			●	0	0	0
			4			●	0	0	0
		2	1			●	6	14	0
		6	1			●	0	0	0
			2			●	0	2	0
			3			●	0	5	0
			4			●	0	0	0
			5			●	0	0	0
			6			●	0	0	0
		7	1			●	0	0	0
			2			●	0	0	0
			3			●	0	0	0
			4			●	0	2	0
			5			●	0	0	0
			6			●	0	0	0
			7			●	0	0	0
			8			●	0	0	0
			9			●	0	0	0
			10			●	0	0	0
Sep. 7	Summary	Trees	Terminals				Infestation Rate		
		4	21	0	0	21	1/21	5/21	0/21

Appendix D2

Numerical evaluations of trees and 1 hibiscus shrub treated with either thiamethoxam or imidacloprid on 28 September 2000. The level of infestation on each branch terminal or node was determined by estimating the total number of live and dead hibiscus mealybugs to 1 of 3 infestation categories (1-25, 26-10, >100). The total number of live hibiscus mealybugs (crawlers, nymphs, adults) was recorded for each sample.

Sample Date	Days Post-treatment	Tree I.D.	Terminal No.	Infestation Category			Number Alive		
				1-25	26-100	>100	Crawlers	Nymphs	Adults
Oct. 26	28	Holtville Hibiscus Treated with thiamethoxam	1			●	0	0	0
			2	●			0	0	0
			3		●		0	0	0
			4	●			0	0	0
			5	●			0	0	0
			6			●	0	0	0
			7		●		0	0	0
			8	●			0	0	0
			9	●			0	0	0
			10	●			0	0	0
			11			●	0	0	0
			12	●			0	0	0
			13	●			0	0	0
			14		●		0	0	0
			15	●			0	0	0
			16	●			0	0	0
			17	●			0	0	0
			18	●			0	0	0
			19			●	0	0	0
Summary		Holtville Hibiscus	Total Terminals	Subtotal Terminals			Infestation Rate		
			19	12	3	4	0/19	0/19	0/19

Sample Date	Days Post-treatment	Tree I.D.	Terminal No.	Infestation Category			Number Alive		
				1-25	26-100	>100	Crawlers	Nymphs	Adults
Oct. 26	28	Holtville Carob Tree Treated with thiamethoxam	1		●		6	3	2
			2		●		0	0	0
			3		●		0	0	0
			4			●	5	4	0
			5	●			0	0	0
			6		●		0	3	1
			7			●	4	12	3
Summary		Holtville Carob Tree	Total Terminals	Subtotal Terminals			Infestation Rate		
			7	1	4	2	3/7	4/7	3/7

Sample Date	Days Post-Treatment	Tree I. D.	Terminal No.	Infestation Category			Number Alive		
				1-25	26-100	>100	Crawlers	Nymphs	Adults
Oct. 26	28	Hashim's South tree Treated with imidacloprid	1		●		0	3	0
			2		●		3	5	0
			3		●		0	1	1
			4	●			0	0	0
			5	●			0	0	0
			6		●		0	0	0
			7	●			0	0	0
Summary		Hashim's South tree	Total Terminals	Subtotal Terminals			Infestation Rate		
			7	3	4	0	1/7	3/7	1/7

Sample Date	Days Post Treatment	Tree I.D.	Terminal No.	Infestation Category			Number Alive		
				1-25	26-100	>100	Crawlers	Nymphs	Adults
Oct. 26	28	Hashim's North tree Treated with imidacloprid	1		●		4	2	0
			2	●			0	0	0
			3	●			0	0	0
			4		●		0	0	0
			5	●			0	1	1
			6	●			0	0	0
			7	●			0	0	0
Summary		Hashim's North tree	Total Terminals	Subtotal Terminals			Infestation Rate		
			7	5	2	0	1/7	2/7	1/7

Sample Date	Days Post-treatment	Tree I.D.	Branch	Node	Infestation Category			Number Alive		
					1-25	26-100	>100	Crawlers	Nymphs	Adults
Nov. 8	41	Hashim's South Tree Treated with imidacloprid	1	1		●		0	0	0
				2	●			0	0	0
				3			●	0	0	0
				4		●		0	0	0
				5		●		0	0	0
				6			●	0	0	0
				7		●		0	0	0
			2	1			●	20	0	0
				2		●		0	0	0

				3		●		0	0	0
				4			●	0	0	0
				5			●	0	0	0
				6			●	0	0	0
				7			●	0	0	0
				8		●		0	0	0
				9		●		0	0	0
				10		●		0	0	0
			3	1		●		0	0	0
				2			●	0	0	0
				3		●		0	0	0
				4			●	0	0	0
				5			●	0	0	0
				6			●	0	0	0
				7			●	0	0	0
			4	1		●		0	0	0
				2		●		0	0	0
				3	●			0	0	0
				4		●		0	0	1
				5	●			0	0	0
				6		●		0	0	1
				7	●			0	0	0
				8		●		2	0	0
			5	1	●			0	0	0
				2		●		0	0	0
				3		●		0	0	0
				4		●		0	0	0
				5	●			0	0	0
			6	1	●			0	0	0
				2		●		0	0	0
				3			●	0	0	0
				4			●	0	0	0
				5			●	0	0	0
Nov. 8	41	Hashim's South Tree Treated with imidacloprid	6	6	●			0	0	0
				7	●			0	0	0
				8			●	0	0	0
			7	1		●		0	0	0
				2	●			0	0	0
				3	●			0	0	0
				4		●		0	0	0
				5	●			0	0	0
				6	●			0	0	0
				7		●		0	0	0
				8			●	0	0	0
				9			●	0	0	0
				10			●	0	0	0
Summary		Hashim's South tree	Total branches	Total nodes	Subtotal Nodes			Infestation Rate		
			7	55	13	23	19	2/55	0/55	2/55

Sample Date	Days Post-treatment	Tree I.D.	Branch	Node	Infestation Category			Number Alive		
					1-25	26-100	>100	Crawlers	Nymphs	Adults
Nov. 8	41	Hashim's North Tree Treated with imidacloprid	1	1			●	0	0	0
				2		●		0	0	0
				3			●	0	0	0
				4			●	0	0	0
				5			●	0	0	0
				6			●	0	0	0
				7			●	0	0	0
				8			●	0	0	0
				9			●	0	0	0
				10			●	0	0	0
			2	1		●		0	0	0
				2		●		0	0	0
				3		●		0	0	0
				4		●		0	0	0
				5		●		0	0	0
			3	1			●	0	0	0
				2			●	0	0	0
				3			●	5	0	0
				4		●		0	0	0
				5			●	0	0	0
				6			●	0	0	0
				7			●	0	0	0
				8			●	0	0	0
				9			●	0	0	0
Nov. 8	41	Hashim's North Tree Treated with imidacloprid	4	1			●	0	1	0
				2			●	0	0	0
				3			●	0	0	0
				4			●	0	0	0
				5			●	0	0	0
				6			●	0	0	0
				7			●	0	0	0
				8			●	0	0	0
			5	1			●	0	0	0
				2			●	0	0	0
				3			●	0	0	0
				4			●	0	0	0
				5			●	0	0	0
				6			●	0	0	0
				7			●	0	0	0
				8			●	0	0	0
			6	1			●	0	0	0
				2			●	0	0	0
				3		●		0	0	0
				4		●		0	0	0
				5		●		0	0	0
				6			●	0	0	0
				7			●	0	0	0
			7	1		●		0	0	0
				2		●		0	0	0
				3		●		0	0	0
				4			●	0	0	0

				5			●	0	0	0
				6		●		0	0	0
				7			●	1	0	0
				8			●	0	0	0
			8	1			●	0	0	0
				2			●	0	0	0
				3		●		0	0	0
				4		●		0	0	0
				5			●	0	0	0
			9	1			●	0	0	0
				2		●		0	0	0
				3			●	0	0	0
				4		●		0	0	0
				5			●	0	0	0
				6		●		0	0	0
				7			●	0	0	0
				8			●	0	0	0
				1		●		0	0	0
Nov. 8	41	Hashim's North Tree Treated with imidacloprid	10	2		●		0	0	0
				3		●		0	0	0
				4			●	0	0	0
				5			●	3	0	0
				6			●	0	0	0
				7			●	0	0	0
			11	1			●	0	0	0
				2		●		0	0	0
				3			●	0	0	0
				4			●	0	0	0
				5			●	0	0	0
				6			●	0	0	0
			12	1			●	0	0	0
				2			●	0	0	0
				3			●	0	0	0
				4			●	0	0	0
				5		●		0	0	0
				6			●	0	0	0
				7			●	0	0	0
				8			●	0	0	0
Summary		Hashim's North Tree	Total Branches	Total Nodes	Subtotal Nodes			Infestation Rate		
			12	89	0	24	65	3/89	1/89	0/89

Sample Date	Days Post-treatment	Tree I.D.	Branch	Node	Infestation Category			Number Alive		
					1-25	26-100	>100	Crawlers	Nymphs	Adults
Nov. 10	43	Camacho's Place No. 4 Treated with thiamethoxam	1	1			●	0	0	0
				2			●	0	0	0
				3			●	0	0	0
				4			●	0	0	0
				5			●	0	0	0
				6			●	0	0	0
				7			●	0	0	0

				8			●	0	0	0
			2	1			●	0	0	0
				2			●	0	0	0
				3			●	0	0	0
				4			●	0	0	0
				5			●	0	0	0
				6			●	0	0	0
				7			●	0	0	0
				8			●	0	0	0
				9			●	0	0	0
Nov. 10	43	Camacho's Place No. 4 Treated with thiamethoxam	2	10			●	0	0	0
				11			●	0	0	0
			3	1			●	0	0	0
				2			●	0	0	0
				3			●	0	0	0
				4			●	0	0	0
				5			●	0	0	0
				6			●	0	0	0
				7			●	0	0	0
				8			●	0	0	0
				9			●	0	0	0
				10			●	0	0	0
			4	1			●	0	0	0
				2			●	0	0	0
				3			●	0	0	0
				4			●	0	0	0
				5			●	0	0	0
				6			●	0	0	0
				7			●	0	0	0
				8			●	0	0	0
				9			●	0	0	0
				10			●	0	0	0
			5	1			●	0	0	0
				2			●	0	0	0
				3			●	0	0	0
				4			●	0	0	0
				5			●	0	0	0
				6			●	0	0	0
				7			●	0	0	0
				8			●	0	0	0
				9			●	0	0	0
				10			●	0	0	0
Summary		Camacho's Place No. 4	Total Branches	Total Nodes	Subtotal Nodes			Infestation Rate		
			5	49	0	0	49	0/49	0/49	0/49

APPENDIX E

COOPERATIVE PROGRAM FOR THE CONTROL OF PINK HIBISCUS MEALYBUG IN IMPERIAL COUNTY

PROGRAM GOAL

The goal of this program is to develop and implement a biologically based, sustainable approach to control and suppress populations of pink hibiscus mealybug (PHM) (*Maconellicoccus hirsutus*) in Imperial County. To accomplish this, a cooperative PHM management team has been formed comprised of representatives from the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) and Agricultural Research Service (USDA-ARS); University of California (UC); California Department of Food and Agriculture (CDFA); and the Imperial County Agricultural Commissioner (ICAC). Other members may be added at a later date.

PROGRAM COMPONENTS

The PHM cooperative program components are: a) administration and coordination, b) public information and awareness, c) survey (monitoring and mapping), d) control program implementation, e) research, and f) evaluation.

Administration and Coordination. The ICAC will provide administrative support for the program and assign a program coordinator. The coordinator will be responsible for ensuring effective communication is maintained among program representatives. Cognizant of the limited resources available, the program coordinator will identify opportunities to share resources to prevent duplication of effort and maintain maximum efficiency.

Public Information and Awareness. The ICAC, in cooperation with CDFA and UC, will conduct an outreach program to inform the public about PHM, the potential impacts of the pest, and what they can do to prevent spread. The outreach program includes, but is not limited to, distribution of informational flyers or pamphlets, newspaper articles or presentation to local groups.

Survey. Countywide survey for PHM will be conducted by CDFA in cooperation with ICAC. Survey data will be utilized to map and identify the spread of PHM. Comprehensive surveys will enable program staff to efficiently implement effective control measures in a timely manner. Furthermore, surveys will provide valuable information needed for distribution of biological control agents, and for researchers investigating the dispersal patterns and potential of PHM. Scientists from CDFA and ICAC will develop survey protocols and procedures. Results of the surveys will be reviewed on a regular basis and adjustments or revisions will be made accordingly.

Control Program Implementation. CDFA will be responsible for the implementation of a biologically based, sustainable control and suppression program. USDA-APHIS will be a major cooperator, providing funds, in-kind support and natural enemies to initiate or supplement the establishment of biological control of PHM. The biologically based, sustainable approach will require mass rearing, release, and evaluation of natural enemies. USDA-APHIS will provide additional natural enemies if needed and available from insectary facilities in Puerto Rico and St. Thomas. If required, natural enemies will be held and evaluated at the USDA-APHIS quarantine facilities in Mission, Texas prior to release in California.

Other control measures will be implemented if they enhance and/or do not interfere with the activity of natural enemies under field conditions. For example, the lady beetle (C*ryptolaemus montrouzieri*) may be utilized to reduce isolated, high level infestations of PHM. In addition, research may identify new or conventional crop protectants that will control PHM and be compatible with natural enemies. Maximum control and suppression of PHM will be achieved through an integrated methods approach utilizing compatible PHM control measures whenever possible.

Research. The UC, in coordination with USDA-ARS, are primarily responsible for conducting research that will enhance the cooperative effort to control and suppress PHM. Scientists from UC and USDA will work closely with scientists from CDFA, USDA-APHIS, and ICAC to assure research objectives are consistent with overall program goals. A number of major research objectives have been identified at this time. These objectives are:

1) Determine the potential for damage of PHM to selected plants grown by the California nursery industry. This includes the description of biology and life history of PHM under Imperial County conditions, determination of host plant preferences and PHM fitness, and key plant species of critical importance to the California nursery industry.

2) Find, collect and evaluate, under quarantine, new natural enemies of PHM. If new natural enemies are identified, rearing, releasing and evaluation activities will be initiated in conjunction with the CDFA program if resources and facilities are available. Priority for this aspect of the program will be based on the most efficacious agent.

3) Study the dispersal and phenology of PHM in the Imperial Valley with emphasis on the development of PHM on various crop hosts.

4) Determine PHM susceptibility to new and currently registered insecticides available to growers.

5) Evaluate candidate chemical controls for efficacy and impact on natural enemies utilized for biological control.

All UC research activities, and CDFA's mass rearing of natural enemies and evaluation of field releases will be conducted in a manner to prevent the accidental introduction of PHM to non-infested areas in Imperial County. This includes establishing temporary facilities in infested areas to conduct program operations.

Program Evaluation. At least two meetings per year will be convened to ensure specific objectives are being accomplished in a timely manner. Meetings will include a review of progress to date, and identification of objectives and resources (staff, equipment, supplies, and funds) required to achieve those objectives. Additional meetings during the year may be necessary. The Program Coordinator will be responsible for notifying cooperators regarding time, place, and agenda items to be discussed.


California Department
of Food and Agriculture